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EDITORIAL

TO BEAT OR NOT TO BEAT

In one of his experiments Galvani placed a frog heart on the back of an electric eel, as long as the eel remained quiet he saw no contractions but when it moved the frog heart contracted violently. Further effects of electric currents on biologic systems were studied soon after these early discoveries of Galvani and Volta and several attempts, some more sophisticated than others, were made during the 19th century to use this new tool in clinical practice. The first artificial pacemaker was built by Hymans in 1932, it weighed 7.2 kg and had to be wound up every 6 min to keep the generator spinning. The first miniature artificial pacemaker was implanted subcutaneously in 1958 by Senning at the Karolinska Hospital in Stockholm, this patient is still alive and in a good condition. After some years, when it was found that the electrode could be attached to the endocardium of the apical part of the right ventricle with the wire introduced through a vein, thoracotomy became unnecessary and the operation is now a minor procedure which can be performed in local anesthesia. This makes it available to very old patients and patients with an acute myocardial infarction (AMI)—two categories which do not tolerate a thoracotomy. If there are no major contraindications, what then are the indications for implantation of an artificial pacemaker?

The indications were discussed and the incidence of pacemaker implantation in different countries was reported at the IV International Symposium on Cardiac Pacing held in Groningen in April 1973. The figures, which refer to the year 1972, were for Japan 6/10⁶ inhabitants, for England 22, for Western Germany 83 and for Sweden 123/10⁶ inhabitants and year. Still higher incidences have been reported from other centres. A different awareness of the clinical picture of Adams–Stokes attacks is a probable explanation to these increasing figures as most centres agree that there are three major indications for pacemaker implantation. 1) bradyarrhythmias including complete heart block, 2) Adams–Stokes attacks, and 3) tachyarrhythmias.

1) *Complete heart block* is one of the relevant arrhythmias in which the prognosis quo ad vitam is best known. Since a majority of patients with a complete heart block nowadays immediately receive an artificial pacemaker a prognostic study must refer to the period before routine pacemaker treatment. A material comprising 193 patients with complete heart block was collected from Malmö during the years 1951–64. As the city of Malmö (221 700 inhabitants in 1958) is served by only one hospital, it may be concluded that this material includes almost all the patients in the town known to have complete heart block. It was found that half of the 193 patients died not more than one year after diagnosis. The excess 1 year mortality remained fairly constant up to the age of 79 years but rose sharply from 40 to 56% in patients aged 80 or more (3).

The life expectancy in patients with *bradyarrhythmias other than complete heart block* is less well known but common to many of them is the variety of symptoms. It is remarkable, however, that in the complete heart block material only 5% of the patients consulted a doctor on account of a slow pulse rate (3); syncope was the cause of consultation in 20%, other cardiac symptoms in 50% and non-cardiac disease in the remaining patients.

2) *The Adams–Stokes syndrome*, defined as attacks of acute cerebral ischemia on the basis of a sudden change in cardiac rhythm, is more common than many published reports would seem to suggest, since many of these patients are given an erroneous diagnosis, often of cerebrovascular disease or epileptic fits. (In Edhag's material (1), 12% had been given a diagnosis of

epilepsy) An Adams–Stokes attack may be impossible to distinguish clinically from an epileptic fit. The differential diagnosis between Adams–Stokes attacks and various neurologic diseases can be very difficult and it should be borne in mind that an Adams–Stokes attack may be accompanied by focal neurological symptoms.

The only way of distinguishing between these diseases is to observe the heart activity during an attack. The patient himself or members of his family can sometimes be taught to palpate the pulse reliably even in the dramatic situation often created by an Adams–Stokes attack. Another possibility is ECG monitoring, either in a coronary care unit or with a telemeter unit on an ambulant patient in the hospital. A portable tape recorder is a good diagnostic tool and saves money because the patients, instead of being confined to hospital, can stay in their normal environment. This is especially useful in patients with long intervals between their attacks. The tape recorder will also reveal if the attacks are produced by a bradyarrhythmia, indicating pacemaker implantation, or a tachyarrhythmia, calling for antiarrhythmic medication

The prognosis quo ad vitam in patients with ms–Stokes attacks is not definitely settled. In aterial comprising 42 patients from the period 950–59 only half the patients survived for one year after their first attack (2). These 42 patients undoubtedly represent a selection because they comprise the group in whom the diagnosis had been verified electrocardiographically or by pulse palpation during an attack. Even if more benign cases are included, with a concomitant improvement of the prognosis quo ad vitam, there is no doubt that these patients suffer from their attacks of dizziness or syncope and that proper treatment with an artificial pacemaker would remove their feeling of insecurity

3) *Tachyarrhythmias* as an indication for pacemaker implantation concern a minor number of patients. In our experience the most beneficial effect is observed in patients with severe bouts of tachycardia, calling for large amounts of antiarrhythmic drugs and resulting in bradyarrhythmias between the attacks of tachycardia. An artificial pacemaker will keep the heart rate at a satisfactory level despite a heavy increase in the dosage of the drug. Over-driving is reported to have been successful in some cases. Data submitted to the IV International Symposium on Cardiac Pacing further support the value of over driving in certain types of tachyarrhythmias.

Patients with an AMI complicated by atrioventricular block or block of two or three of the main bundles are often subjected to temporary or long-term artificial cardiac pacing. But the initial enthusiasm seems to have vanished and in some of these authors the native hue of resolution is sicklied o er with the pale cast of thought" A survey of the literature reveals an absence of reliable control materials and in the published accounts the authors tend to ignore the pronounced difference in prognosis between for instance, complete heart block as a complication to an anterior rather than a posterior infarction (3). Our indications for artificial pacing in patients with AMI are unsatisfactory response to isoprenaline or atropine in complete heart block or other pronounced bradyarrhythmias with hemodynamic deterioration due to the slow pulse rate. One or more Adams–Stokes attacks in AMI regardless of the type of block, is likewise an indication for pacing.

To what extent is the prognosis improved in patients who are artificially paced with the above mentioned indications? The exact figure cannot be extracted from the published studies for several reasons. To get a true comparison one would have to make a statistically acceptable selection of every second patient for pacemaker implantation while the other received medical treatment. This would be unethical in the light of present knowledge. The 193 patients with complete heart block (3) lend themselves to a comparison, especially if one excludes those with AMI and digitalis intoxication as etiologic factors. This leaves a 1 year survival of 69 % which should be compared with rates of 85–90 % in several paced series (4). The Malmö material has been followed further for the group below 80 years the survival rate in the non-paced patients with complete heart block was after one year 69 % after three years 50 and after five years 38 % while for the paced patients it was 88 67 and 62 % respectively The corresponding figures for non-paced patients aged 80 years or more were 16 11 and 0 % and for paced patients 65 64 and 27 % respectively

It is obvious that high age does not contraindicate artificial pacing. The beneficial effect is apparent not only from the higher survival rate

but also from an improvement in the patients condition. This is not just an addition of years to life but in many instances of life to years. Furthermore, many old patients with confusion and other cerebral symptoms secondary to a slow pulse and asystoles become easier to nurse when the rise in pulse rate restores their cardiac output to normal values. It is important to remember that Adams–Stokes attacks can hide themselves behind ery varied symptoms: patients with syncopal attacks and focal neurologic symptoms, pa tients with fits of an epileptic" type, patients who appear in the emergency wards with iterated concussions, all may have Adams–Stokes attacks as a cause of their symptoms.

An active search program aimed at finding all possible pacemaker candidates in a defined population would yield a true incidence for pacemaker implantation. Malmö has the advantage of having just one hospital serving the entire population of a quarter of a million inhabitants and such study is in hand there.

There is no doubt that the number of pace maker implantations will increase in the near future as Adams–Stokes attacks are diagnosed more accurately The incidence in Western Ger *many shows a steady increase from 50/10⁶ inhabi*tans in 1970 to 100 in 1972. The corresponding figure in Malmö was 160. These patients have to be checked at regular intervals.

A true incidence figure is of importance for the pacemaker organization but it is also essential to know the number of pacemaker patients in different age groups. Most patients are in the highest age groups but a comparatively large number are under 70 and in these a nuclear pace maker can be considered. A conventional energy supply is relevant in older patients and in patients with a short life expectancy The search for new energy sources of longe duration than mercury batteries, such as the lithium cell or biological energy sources, is of importance and a true incidence figure would be of value when deciding whether to concentrate on making nuclear power more effective or find other energy sources which are cheaper but of shorter duration.

Probably future development will go along both these lines. New indications for nuclear power will probably emerge, the completely mechanically driven heart as an alternative to heart transplan tation will need such large amounts of energy that conventional energy resources will be insufficient.

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BOOK REVIEW

Lymphocytes Structure production functions By G Astaldi and L. Lisiewicz. 378 pages. 12 000 Lire. Idelson, Naples, Italy 1971

The well known American hematologist Crosby writes in his foreword to the present volume "Twenty-five years ago the lymphocyte was a boring little cell that no one paid much attention to" The reviewer may perhaps add that 45 years ago, when I began in medical school, the big feud between the giants of histology—Pappenheim, Maximov Ferrata, on the one hand, and Ehrlich and Nägeli on the other—was still raging. My beloved teacher Hammar in Uppsala, who was decidedly the greatest authority on the thymus, and his pupil Hellman in Lund, the foremost investigator of the lymphatic organ, were much engaged in these discussions. The rt of the matter was already at that time con- ed with the problem is the lymphocyte a pluri- tential stem cell or is it just a boring little cell"? During the last two decades it has become evident that the lymphocyte or the two lymphocytes T and B, because there is ample evidence that there is not only one type of this cell, have many and fundamental functions. It is appropriate that Astaldi, the pupil of Ferrata, the leader of the famous Italian school of hematology should write a book about these problems. It was published just before the lymphocyte problem became really hot—the idea about T and B cells is of very recent origin—but still the volume is a gold mine of information. Astaldi has contributed knowledge in many fields and his discussions of his own work and of other contributors results are therefore well balanced.

In some ways it is even an advantage that the volume has been written before the great problem of thymus and bone marrow lymphocytes was clearly defined. This gives the reader a non-biased review of the situation at the moment when this discussion started. Cronkite has spoken about the "ubiquitous lymphocyte" The reader gets a vivid picture of the lymphocytic involvement in immunology its connection with plasma cells and its importance for delayed hypersensitivity as well as the many problems of organ transplantation, where this little cell plays the first violin in the orchestra.

The Astaldi school has made many contributions to the problem of lymphocyte activation and also inhibition. This explains why a great part of the volume is dedicated to a discussion of phytohemagglutinating agents. The clinician who consults the chapter on lymphocyte inhibitors may be somewhat disappointed by the fact that so many data are derived from in vitro examinations of cells, whereas the clinical studies are more frugal.

On the whole it may be said that this volume devoted to one of the most central biological problems in modern medicine should be of great interest to many specialists. Surgeons (transplantation), internists, allergists with different interests, dermatologists as well as the basic investigators may profit greatly from a study of Astaldi's work. It is therefore recommended to medical libraries and to special investigators alike. The volume is well illustrated, also with a number of excellent colour pictures.

Jan G Waldenström

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FAMILIAL THYROXINE BINDING GLOBULIN DEFICIENCY

A Study of Three Danish Families

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Abstract. Three Danish pedigrees have been studied with view to reduced thyroxine-binding globulin (TBG). The study confirmed that this defect is transmitted by X-chromosome-linked dominant inheritance, there being no man-to-man transmission, but also that it may occur in two types: deficient TBG and low non-zero TBG. Definite changes of TBG and thyroid function tests were found in the hemizygous men, whereas the heterozygous omen showed marked variation in the range between the values for hemizygous men and normal per sons. Determination of total thyroxine appears to afford more guidance than the protein-bound iodine suggested by others. T of four omen on oral contraception exhibited such deviating TBG values that they are assumed to be TBG heterozygous. I all persons free thyroxine proved normal or slightly reduced but all the persons tested are euthyroid. However the propositus of one family had previously been treated for Graves' disease.

Tanaka and Starr (13) in 1959 described the first case of thyroxine-binding globulin (TBG) deficiency in an euthyroid male. The first genetic study of TBG deficiency was published in 1965 by Nicoloff et al. (6) who assumed that the defect was transmitted by autosomal dominant inheritance. This view was altered 2 years later by the study of Nikolai and Seal (9) who concluded that the heredity was X-chromosome-linked dominant. Divergences in this respect were due partly to the inability to rule out a man-to-man transmission as the most important criterion of Nikolai and Seal's theory and partly to the fact that many males did not exhibit a total lack of TBG. On the basis of, inter alios, the investigations made by Refetoff et al. (10), it is the prevailing opinion to-day not only that the theory of Nikolai and Seal is correct, but that the spectrum of TBG abnormalities encountered in humans may be explained as a mutation in a single gene locus controlling TBG synthesis.

Most papers on TBG deficiency are from Anglo-Saxon countries. So far only one family study has come from Scandinavia (2).

The result of the first genetic study from Denmark will be published below. It comprises three families. The marked variation in TBG concentration cannot but influence the evaluation of several thyroid variables, a problem which is also apparent from the present study.

METHODS

Serum. TBG as determined at the Medical Laboratory, Copenhagen, by the method of Nielsen et al. (7). The concentration is stated in arbitrary units (a.u.). Thyroxine-binding prealbumin (TBPA) was determined in the same laboratory by the immunochemical method of Laurell (4). Other methods were as follows: protein-bound iodine (PBI) using Technicon AutoAnalyzer, thyroxine displacement ($T_4(D)$) by means of commercial kit (Abbott Tetrasorb® 125), free thyroxine (FT_4) by the method of Sterling and Brenner (12), radio-triiodothyronine uptake (RT_3U) using commercial kit (Abbott Triosorb® 125) and stated in % of reference serum. Protein electrophoresis on paper was performed to rule out the presence of other abnormal protein findings in the serum. The abbreviations are those suggested by the American Thyroid Association (11).

Clinical procedure. Each of the three propositi put the author into touch with their families, whose members received questionnaire asking about their present health, past or present thyroid disorders, and current medication, if any. Spouses were asked about thyroid disease, but were otherwise not included in the study. After the questionnaires had been returned, all persons wer asked to attend except infants under 6 months of age. In children under 10 years of age the TBG, $T_4(D)$, and FT were determined if it was possible to obtain sufficient serum.

Fig. 1. Pedigree of family J.P. with TBG deficiency.

Fig. 2. Pedigree of family A.A. with low non-zero TBG.

MATERIAL

Pedigree J.P. The propositus (II 5) was admitted for examination when, in a routine investigation, the PBI was found to be reduced. Physical examination showed no abnormalities. Moreover the BMR and 4-hour ^{131}I-uptake were normal. Case II. 6, sister of II. 5, is on substitution therapy for Addison's disease. The spouse of III. 4 was the only one of the family who had

Table I. *Serum studies in family J P with TBG deficiency (affected males)*

Case no.	Age (y.)	RT U (%)	PBI (μg/100 ml)	T_4(D) (μg/100 ml)	FT (ng/100 ml)	TBG (a.u.)	TBPA (g/1000 ml)
II.2	64	137	4 0	1.3	0.90	0	0 25
II 3	62	141	2.0	1.8	0.80	0	0.28
II.5	57	188	2 0	1.5	1 17	0	0.24
III 3	34	147	1.1	1.0	0.70	0	0.26
III 11	26	109	2 0	2.2	2.11	0	0 25
III 13	27	187	2.1	1 7	1 10	0	0.22
IV 10	2	—	—	—	—	0	—
Normal range		79–121	3.5–7.5	3.5–6 9	♂ 0.7–3 0 ♀ 1.0–3.1	68–140	0.12–0.39

history of thyroid disease (Graves' disease), but normal TBG and function test. Cases III. 5 and III 13 were married to each other (Fig. 1.) Table I gives the results of member of thyroid function tests. A total of 37 per sons (17 women, 20 men) in the age range 2–92 years were examined.

Pedigree E.N The propositus (I. 4) was admitted be cause of suspicion of arterial hypertension. He complained of nervousness and palpitations, but was clinically euthyroid There was no evidence of phaeochromocytoma. Cholesterol, BMR, and 24-hour ^{131}I-uptake were normal. PBI and RT$_3$U did not differ from the values from the first TBG determination in 1972, listed in Table III. Within this family 6 males (1 affected, 5 normal) and new born girl were tested.

Pedigree A.A The propositus (I. 1) had been treated as an outpatient for Graves' disease at the age of 60. Diabetes mellitus was detected at the same time and treated by dietetic measures and tolbutamide. At the age of 71 he was admitted with recurrence of his thyrotoxicosis. Treated first with methimazole, but owing to increasing goitre later also with thyroid. The medication was discontinued 2 years later and since then the patient has been euthyroid. At admission PBI as 7.2 μg/100 ml, RT$_3$U 285% and BMR +29% The new thyroid analyses done in connection with the TBG determination are apparent from Table IV Thirty members of the family were investigated, hereas 10 either refused to participate, are newborn, or were living abroad. (Fig. 2.)

RESULTS

Pedigree J.P From Fig. 1 it is apparent that man-to-man transmission did not occur The daughters of hemizygous fathers were carriers in all cases but one (III. 6). The results of the TBG TBPA and thyroid function tests in 14 members of the family are given in Tables I and II. The remaining 23 persons were normal. Case IV 2 had a borderline TBG value, but must be considered heterozygous owing to the low PBI and T_4(D). FT_4 was normal or slightly reduced in both sexes, showing a median value at the lower end of the reference range (males 1 13 females 1 06 entire family 1.37).

Pedigree E.N The laboratory analyses are given in Table III. The propositus had a low non-zero TBG concentration. His FT_4 was 0.82 as compared to the family mean of 1 42.

Pedigree A.A The laboratory results for the 12 persons showing abnormal TBG are presented in Table IV All the men had a low non-zero TBG. *In this family too, there was no man-to-man* transmission. Two hemizygous men had normal daughters, whereas no data are available for the

Table II. *Serum studies in family J P with TBG deficiency (affected females, "carriers")*
Normal values, see Table I

Case no.	Age (y)	RT U (%)	PBI (μg/100 ml)	T_4(D) (μg/100 ml)	FT (ng/100 ml)	TBG (a.u.)	TBPA (g/1000 ml)
I.2	92	112	3.3	2 0	0.96	50	0 16
II 6	52	109	4 5	3 0	0 93	55	0.26
III 1	41	112	3.5	2.5	1.50	47	0.20
III 4	31	146	3 2	2.7	1 10	36	0.24
III.5	25	164	2 9	1 9	0 80	29	0 24
IV 2	13	115	3.1	2.5	—	68	0.16
IV 11	2	—	—	—	—	48	—

Table III. *Serum studies in family E. N with low non-zero TBG*

Normal values, see T ble I

Case no	Sex	Age (y.)	RT U (%)	PBI (μg/100 ml)	T_4(D) (μg/100 ml)	FT (ng/100 ml)	TBG (a.u.)	TBPA (g/1000 ml)
I. 1	♂	73	—	5.1	6.2	1.77	101	—
I. 2	♂	71	101	5.9	6.2	—	93	—
I 3	♂	68	100	17 1	5.7	1 77	103	—
I 4*	♂	62	147	2.5	1.9	0 82	23	—
II 1	♂	33	113	5.3	5.3	1.25	97	—
III 1	♂	5	—	—	5.2	1.30	109	—
III. 2	♀	2	—	—	—	—	—	—

Affected.

father of two sisters (I 4). The Table is divided into three groups, (A) hemi- and heterozygotes, (B) suspected heterozygotes and (C) hormone induced elevation of TBG Cases II. 4 II. 5 III 16 and III. 18 are on oral contraception, II. 8 is being treated with a combined androgen-oestrogen preparation and II 11 is pregnant.

All pedigrees. Serum protein electrophoresis proved normal in all persons studied. TBPA, determined only in families J P and A.A. was ound to be normal.

DISCUSSION

The transmission pattern of TBG deficiency in these three Danish pedigrees is compatible with X-chromosome-linked heredity None of the families showed a man-to-man transmission, and most daughters of affected fathers are heterozygous. It is apparent from this family study that the TBG defect may occur in the form of two types: deficient TBG (type D) and low non-zero TBG (type L) (cf Refetoff et al. (10)). Pedigree J.P is type D and pedigrees E.N and A.A. are type L.

In heterozygous women the TBG concentration is usually about half the value seen in normals, but with a marked variation in the range between those of hemizygous men and normal persons. Indeed, this is apparent also from the present study This distribution of the TBG values in heterozygous women is compatible with Lyon's (5) hypothesis of randomized and per

Table IV *Serum studies in family A A with low non-zero TBG*

Normal values, see Table I

Case no.	Sex	Age (y.)	RT U (%)	PBI (μg/100 ml)	T (D) (μg/100 ml)	FT (ng/100 ml)	TBG (a.u.)	TBPA (g/1000 ml)
A. Affected								
I 1	♂	75	169	4.0	3.0	3.35	19	—
II. 3	♀	45	122	3.4	3.0	—	68	—
II:10	♂	36	128	2.9	2.3	—	15	—
II 12	♂	25	142	2 5	1.8	1 10	36	—
III 6	♂	9	—	2.2	—	—	36	—
III 7	♂		—	—	—	—	20	—
B. Suspected affected on oral contraception								
II 4	♀	24	97	4 6	3.4	0.10	74	0 23
II 5	♀	22	104	5.9	5.2	0.10	77	0 28
C. Normals, pregnant or on hormonal treatment								
II 8	♀	49	73	6.0	5.7	2.00	185	0.25
II 11	♀	29	57	8.5	8.0	1.30	295	0.16
III. 16	♀	22	56	—	7.8	2.00	213	0.24
III 18	♀	19	76	7 1	6.7	1.60	150	—

manent inactivation of one X-chromosome in the early foetal development of the female somatic cells.

An attempt has been made to map the locus of the TBG gene on the X-chromosome by means of red–green colour vision and Xg^a red cell antigen (10).

The defect of TBG synthesis is specific, benign, and cannot be correlated to any clinical disease or other inherited abnormality (8, 9 10).

In the great majority of cases the reduced TBG concentration is demonstrated accidentally owing to a dissociation of the values for PBI and RT_3U (8). In the case of heterozygous women a determination of these two variables is often not sufficient to disclose the condition in these euthyroid women (cf Tables II and IV). According to the present studies T (D) seems to be lower and therefore a more reliable measure of the defect than the simultaneously determined PBI.

Diagnostic problems arose in only one case (propositus A.A.), admitted in a thyrotoxic state with a normal PBI and a high RT_3U at a time when TBG could not be determined.

In pedigree A.A. six women are on hormonal medication, of whom two sisters (II. 4 and II. 5) differ so much from the others in the results for TBG T_4(D) and RT U that they must be assumed to be TBG heterozygous (cf Table IV). The values accord with those found by Nikolai and Roberts (8) in women on oestrogen medication, on which heterozygous women have low normal TBG whereas normal women show high-normal to elevated TBG

FT determined in all three families, proved to be normal or slightly reduced, as also reported by others (1 2, 3 8 10) in persons with TBG deficiency

No member of our families had previously had thyroid function tests Since all are clinically euthyroid, the affected persons and their general practitioners have been informed of the defect to avoid possible unnecessary treatment.

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THE VALUE OF THE EFFECTIVE THYROXINE RATIO COMPARED WITH OTHER COMMON THYROID TESTS

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Abstract. The purpose of the present work was to compare the reliability of the new in vitro thyroid test, ETR, with that of the other commonly used thyroid tests in three different ways. 1. Comparison of the test results with the clinical picture obtained by a doctor on the basis of anamnesis and status. 2. Investigation of the correlation of ETR with the other thyroid tests. 3. Investigation of the ability of the tests to discriminate between states of hypo-, hyper- and euthyroidism. The material examined consisted of 62 patients. The results showed ETR to be the best of the laboratory tests according to all three criteria. ETR correlated with the clinical picture obtained on the basis of anamnesis and status in 95% of the cases. ETR correlated highly significantly with the other thyroid tests used (T T (= T uptake), FT index, PBI, cholesterol and radioiodine test). The discriminative capacity of ETR was highly significant in all the states, while the ability of the T test to distinguish hypothyroidism was not statistically significant.

Mincey et al. (6) described in 1972 a new type of in vitro thyroid test, ETR (the effective thyroxine ratio), which is technically simpler than the assay of free serum thyroxine (7) or the calculation of indices for it (1, 3, 4). ETR provides a reliable indication of the thyroid status and is independent of the variations in concentration of the serum protein binding thyroid hormone (9), which occur e.g. during pregnancy and during the use of oral contraceptives. The ETR test assays simultaneously the serum total thyroxine and the thyroxine-binding capacity of thyroglobulin (TBG) (6). The result is expressed as a ratio to normal serum. It has been shown that ETR has a 99% diagnostic accuracy in determining the thyroid status of a material of patients consisting of eu-, hyper- and hypothyroid individuals, gravidae, and users of contraceptives (9).

The purpose of the work was to compare the value of ETR with the other in vitro thyroid tests and the radioiodine test.

MATERIAL AND METHODS

The material consisted of 62 patients, 36 of whom were euthyroid, 16 hyperthyroid and 10 hypothyroid. The thyroid status was clinically evident and the material included no patients with other diseases of the thyroid gland, no gravidae, and no users of contraceptives. From each patient 10 ml venous blood was drawn without anticoagulants and stored at +4°C until analysed.

ETR was assayed according to Mincey et al. by the Res-O-Mat® ETR test (6). The method is a competitive protein binding assay and a resin strip is used as the secondary binder. The results have been given as a ratio to normal serum, the normal values confirmed by numerous investigations being 0 86–1 10. Between-assay variation was 5.2%. Thyroxine (T_4) was assayed by the Res-O-Mat® T test (5), and the results are here expressed as µmol of thyroxine iodine/l. The normal values are 0.26–0 70 µmol/l. T uptake was measured by the Res-O-Mat® T test (7). The T results were calculated in a manner inverse to the original method, and the normal values are therefore logical, i.e. 0 85–1.20. Between-assay variation was 4 % for T and 8 3% for T. Free thyroxine index (FT index) is a ratio calculated from the T and T assays. Its normal values are 4 4 15.7. Protein-bound iodine (PBI) was assayed by the wet ashing method (2). The results are expressed as µmol/l, the normal values being 0 27–0 63 µmol/l. Total cholesterol was assayed according to Pearson et al. (8). The normal values for it are 4 2–7 8 mmol/l. The ETR, T and T assays were measured on an automatic γ-counter (GTL-300 Wallac) with on-line connection to an electronic computer (Sony Sobax 2700E). In the radioiodine test a dose of 10–25 µCi of ^{131}J was given per os and the uptake and output values were measured after 24 hours with a S.A.L.P. medical spectrometer SPM3. The normal values for radioiodine uptake are 20–45%, and those for radioiodine output 40–65%.

Table I. *The reliability of the tests in the different states expressed in % and the proportion of errors. Status and anamnesis are used as reference*

	Euthyroid		Hypothyroid		Hyperthyroid		Combined material	
ETR	2/36	94.4	1/10	90.0	0/16	100.0	3/62	95.2
FT index	2/36	94.4	3/10	70.0	3/16	81.3	8/62	87.1
T	6/36	83.3	3/10	70.0	1/16	93.8	10/62	83.9
RaI uptake	4/29	86.2	4/9	55.3	1/14	92.8	9/52	82.7
RaI output	4/29	86.2	6/9	33.3	2/14	85.7	12/52	76.9
T uptak	4/36	88.8	6/10	40.0	7/16	56.2	17/62	72.6
PBI	9/34	73.5	5/10	50.0	2/16	87.5	16/60	73.3
Cholesterol	4/21	80.0	2/7	71.4	6/9	33.3	12/37	67.6

RESULTS

Table I shows the reliability of the different thyroid tests in evaluating eu- hypo- and hyperthyroidism. The material has been classified according to anamnesis and status. Fig. 1 shows the whole material combined. As will be seen, ETR proved to be the most reliable method (95%); the calculated FT_4 index is the second best (87%) and T_4 and radioiodine share the next position (83%). The other tests yield noticeably poorer results. Cholesterol is the poorest method all, as has already been shown in several works. Indication of euthyroidism (Table I), ETR FT_4 index have a reliability of 94% T_3 iodine are next best (87%). To reveal pothyroidism, ETR is by far the most reliable method (90%) the other techniques being clearly poorer According to the present results cholesterol is still a fairly reliable method for screening hypothyroidism (71%). ETR is 100% reliable in revealing hyperthyroidism, and the next best methods are T_4 and radioiodine uptake (93%) followed by PBI (87%). Cholesterol and T_3 proved to be useless as criteria.

According to Table II, ETR correlates highly significantly with all the other thyroid tests used in the work. The *t* values of this Table confirm the mutual order of superiority arrived at on the

Table II. *Correlation of the different tests with ETR*

	N	r	t	p
ETR/FT index	62	0.8671	13.3854	
ETR/T	62	0.7874	9.8940	
ETR/RaI uptake	52	0.8032	9.5331	
ETR/RaI output	5?	−0.7499	8.0162	
ETR/T uptake	62	0.6404	6.4589	
ETR/PBI	60	0.6534	6.5737	
ETR/cholesterol	37	−0.5718	4.1235	

<0.001

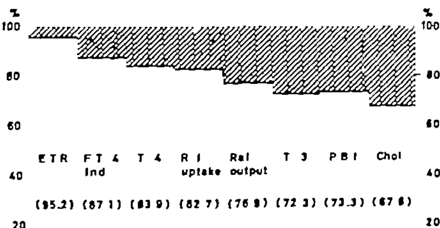


Fig. 1 Reliability values in the combined patient material.

Table III. *Means (X) and standard deviations (S.D.) and p-values in the comparison of the results with euthyroidism*

	Euthyroid			Hyperthyroid			Eu/hyper	Hypothyroid			Eu/hypo
	X	S.D.		X	S.D.		p	X	S.D.		p
ETR	0.949 ± 0.054		36	1.313 ± 0.131		16		0.757 ± 0.090		10	
FT index	10.5 ± 3.4		36	22.9 ± 8.2		16		3.7 ± 2.1		10	
T	0.527 ± 0.160		36	0.942 ± 0.282		16		0.206 ± 0.100		10	
RaI uptake	31.9 ± 10.3		29	71.4 ± 17.7		14		14.3 ± 10.7		9	
RaI output	54.4 ± 11.1		29	15.7 ± 15.3		14		57.2 ± 13.6		9	NS
T uptake	0.967 ± 0.097		36	1.246 ± 0.171		16		0.895 ± 0.102		19	NS
PBI	0.542 ± 0.119		34	1.160 ± 0.355		16		0.296 ± 0.172		10	
Cholesterol	6.25 ± 1.71		21	4.55 ± 0.94		9		9.28 ± 2.87		7	

$p < 0.001$ ** $p < 0.01$ $p < 0.05$, NS $p > 0.05$

basis of Fig. 1 and Table I. Table III shows the means and standard deviations for the different groups of patients. The *p*-values of the Table show how well hypo- and hyperthyroidism can be distinguished from euthyroidism. All the tests employed can discriminate between hyperthyroidism and euthyroidism. T_3 and RaI output, however did not distinguish hypothyroidism significantly from euthyroidism. Fig. 2 shows the results of ETR, RaI test and T_3 (mean ± S.D. and *p*-value) in the different patient groups.

When the anamnesis and the status, the correlation with the other thyroid tests, and the discriminative capacity are taken into account, ETR proves to be by far the best of the thyroid tests employed in this work.

DISCUSSION

The results obtained in this screening experiment are in agreement with other recent ETR findings. Higher reliability values have been obtained in studies of patients who had changes in serum protein pattern (9). The following facts, furthermore, seem to justify the introduction of ETR into routine use. the test is simple to perform, it is not sensitive to iodine contamination, and it yields reliable results despite variations in TBG (e.g. pregnancy oral contraceptives, nephrosis, liver diseases). The FT_4 index calculated from T_4 and T should reach the same level of reliability as ETR, because protein variation is eliminated. The presence of two variables apparently increased the sources of error in the present work. The radioiodine test proved reliable only in discriminating between euthyroidism and hyperthyroidism. The possible iodine contamination, errors in collection and the long duration of the test reduce the value of this method. The T_3 uptake appeared to be an even poorer test than is generally thought. It did not distinguish hypothyroidism from euthyroidism. Cholesterol can still be justly used in the diagnosis of hypothyroidism

The number of thyroid tests has increased dur

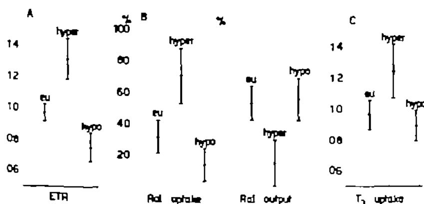


Fig. 2. (*A*) ETR, (*B*) radioiodine tests, (*C*) T uptake (mean ± S.D.) in the different states.

ing the last few years. Clinicians frequently ask for the whole scale available. In such cases the number of contradictory results increases and the final diagnosis becomes difficult, because the tests have not been chosen critically According to the present work ETR is well justified for use in the diagnosis of both hypothyroidism and hyperthyroidism.

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ADRENAL SCINTIGRAPHY IN PRIMARY ALDOSTERONISM

PRELIMINARY REPORT

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When the diagnosis of primary aldosteronism due to adenoma of the adrenal cortex has been established on the basis of adequate biochemical and endocrinological data, the site of origin of the tumour must be established. The tumour is unilateral in about 90% of the cases, and diagnosis as to the side will limit the extent of surgery involved.

W wish to report our experience with the method of Conn et al. (2) in which radioactive cholesterol is administered i. to the patient and the adrenals later visualized by the use of gammacamera.

MATERIAL AND METHODS

The procedure was employed in two female patients who satisfied the biochemical and clinical criteria for primary aldosteronism (Table I). They both had increased plasma aldosterone and aldosterone secretion levels, not suppressed by sodium loading. In subject 2 low and unresponsive plasma renin activity was found, whereas subject 1, with serum creatinine values of 1.6–2.5 mg/100 ml, showed normal postural increase during sodium depletion. Plasma renin activity plasma aldosterone and aldosterone secretion rate are measured as described previously (4 5 6). ^{131}I-19-iodocholesterol as obtained from Phoenix Memorial Laboratory University of Michigan. A dose of 2.2 mCi, dissolved in absolute alcohol and diluted to 10% solution with physiological saline and 0.2% Tween 80, was administered i. to each patient immediately upon receipt of the shipment. Adrenal scintigraphy was performed during 4–10-day period after administration of the dose by gammacamera (Nuclear Chicago, Pho-Gamma III, fitted with parallel hole collimator 360 keV 1 000 holes).

RESULTS

The photoscintigrams and removed adrenal glands are shown in Figs. 1 and 2. The postoperativ decrease in plasma aldosterone as well as the increase in plasma renin activity are shown in Table I. The histological diagnosis was encapsulated adrenal adenoma in both cases. T months after surgery the patients were normotensive with normal serum electrolytes

DISCUSSION

Conventional methods, such as retroperitoneal pneumography and arteriography are not very suitable for the

Table I *Clinical data f the patients*

ASR = aldosterone secretion rate (µg/24 h), PA = plasma aldosterone concentration (pg/ml), PRA plasma renin activity (ng ang. I/ml/h)

Pat. no.	Sex	Age (y.)		ASR (Supine)	PA (Supine)	PRA (Supine)	(Upright)
1	♀	56	Preoperatively				
			Sodium depl.	345	186	0.3	0.9
			Sodium load.	303	205		
			Postoperatively				
			Upright, ambulatory		15		1 6
2	♀	51	Preoperatively				
			Sodium depl.	352	86	0.2	0.2
			Sodium load.	725	108		
			Postoperatively				
			Upright, ambulatory		54		0.8



Fig 1



Fig. 2

Fig. 1 Subject 1. Photoscintigram (posterior view) showing accumulation of radioactivity almost exclusively in the *left* adrenal region, and the removed left adrenal gland with adenoma, 2.2 cm in diameter. The outline of the kidneys, as drawn from results of ¹³¹I-hippuran renography. *Fig. 2*. Subject 2. Tumour on the right side

demonstration of aldosteronomas since most of them are small, less than 2.0 cm in diameter and relatively avascular.

Selective adrenal venography has been employed with success since 1968 (3) and is still used extensively. With this method it has been possible to distinguish between tumour and bilateral hyperplasia (2). The disadvantage of the method is that it needs extensive experience which is not easily obtained. The fragile medullary vessels may rupture during the procedure, thereby producing an intra-adrenal hemorrhage. Adrenal insufficiency has also been reported, as well as accidental cure of Cushing's syndrome and of primary aldosteronism (7).

Determination of the aldosterone content of adrenal venous blood is the method of choice from a functional point of view. A positive finding by this procedure assures that an actively secreting gland is removed. Available methods allow aldosterone to be measured in less than 0.1 ml of adrenal venous blood, due to the very high concentration compared to that of the periphery and to the high sensitivity inherent in the radioimmunoassay technique. The problem with the method is technical and comparable to that of venography. Besides, one cannot be sure that the blood drained through the catheters is entirely of adrenal origin.

The method of Conn et al. (2) with photoscintigraphy of the adrenal glands after administration of ¹³¹I 19-iodocholesterol eliminates the disadvantages inherent in adrenal vein catheterization. The adrenals are detected by scintigraphy some days after administration of the tracer. A concentration ratio between adrenal tissue and extra-adrenal tissue of 400 : 1 is present after one week (1). It is postulated that this ratio is achieved because all internal organs, except the adrenal glands, rapidly de-iodinate iodocholesterol (1). About 50% of the administered dose is excreted during the first two days and the radiation to the total body is calculated at about 1 rad for the standard diagnostic test (1).

In our cases the adrenal scintigraphy was performed for the localization of the tumour. There is evidence that adrenal scintigraphy before and after adrenocortical suppression with dexamethasone might be of value, adding a functional component to the anatomical diagnosis (2). This may be an interesting approach when combined with a functional evaluation by endocrinological assays.

The positive finding of adrenal tumours as described must be considered a most encouraging and significant progress.

Note added in proof ¹³¹I-19-iodocholesterol is now available from Institutt for Atomenergi, Kjeller, Norway.

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ANTIDIURETIC RESPONSES TO HYPERTONIC SALINE INFUSION WATER DEPRIVATION, AND A SYNTHETIC ANALOGUE OF VASOPRESSIN IN PATIENTS WITH HEREDITARY HYPOTHALAMIC DIABETES INSIPIDUS

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Abstract. Eight patients with previously untreated hereditary hypothalamic diabetes insipidus have been investigated Infusion of hypertonic saline produced no significant antidiuresis in any of seven patients tested. In tw however urine osmolality increased to values ex ceeding plasma osmolality During water deprivation (8–18 h) urine osmolality increased in all patients, but only in three were values exceeding plasma osmolality reached. Administration of new long-acting analogue of vasopressin, 1-deamino-8-D-arginine vasopressin (DDAVP), effectively decreased urine volumes and increased urine osmolality in all the patients. The peptide was used for long-term treatment in seven cases. The results are discussed in relation to the pathogenesis of the disease.

Hypothalamic diabetes insipidus is a consequence of lack of the antidiuretic hormone of man and most other mammals, 8-arginine vasopressin (AVP), and may result from any condition which damages the hypothalamic-neurohypophyseal system where the hormone is synthesized and stored (9 26, 27). According to the classification of Randall et al. (25) the disease can be divided into two major categories: primary and secondary Tumours and trauma are known causes of secondary diabetes insipidus (3, 25). The aetiology of primary diabetes insipidus, however is not known, but in a minority of the cases the condition is hereditary (3, 22). Because of the rare occurrence of the hereditary form of the disease, this condition has not been extensively studied. The discovery of a family with hereditary hypothalamic diabetes insipidus, whose members had not previously been investigated or treated, presented an opportunity for further study of patients with this disease. In the present study eight patients were investigated, especially their antidiuretic responses to infusion of hypertonic saline, water deprivation, and administration of vasopressin. A new long-acting analogue of vasopressin, 1-deamino-8-D-arginine vasopressin (DDAVP), was used, not only for diagnostic pur poses, but also for long-term treatment (12–18 months) in seven of the patients.

MATERIAL AND METHODS

The material consists of family with 64 members (Fig. 1) of whom 15 were known to hav thirst and polyuria W investigated 8 of these 15 members; in the other 7 the diagnoses were based on personal interviews or on descriptions given by close relatives.

The patients had certain clinical features in common. Increased thirst and polyuria were regularly recognized in early childhood These symptoms wer ell known in the family and are regarded not as sign of disease but as an insignificant peculiarity of no consequences for the ability to lead normal lif Not even at the age of 15–25 hen the symptoms ere most pronounced and quite disturbing in several cases, had any member of this family asked for medical aid to relieve the symptoms. However in patient 1 the diabetes insipidus was recognized during military service. Therapy with posterior pituitary powder intranasally as instituted, but to obtain an ppreciable reduction of the urine volumes the patient had to take quite large doses. This medication then caused abdominal discomfort and the patient preferred to dispense with treatment after few months.

The degree of thirst and polyuria showed great individual variation, the daily urine output ranging from 2.5 to 15.2 l (T ble I) Many patients showed spontaneous improvement of the symptoms with increasing age. Thus patient 7 reported measured urine volumes of 10–12 l/day in her youth, but now at 72, the urine production seldom exceeded 3 l/day

Hypertonic saline infusion. The hypertonic saline infusion test was performed according to the method of

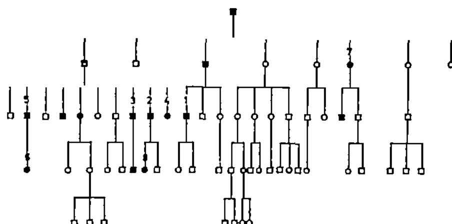


Fig. 1. Pedigree of the family with 64 members of whom 15 had symptoms compatible with hypothalamic *diabetes insipidus*. Figures 1–8 denote the members investigated. □ = male; ○ = female; ■ ● = members with symptoms of disease.

Carter and Robbins (6) with slight modifications. After bladder catheterization the patients were given a water load, 20 ml/kg b.wt. during 1 h, producing a diuresis of 5 ml/min or more. An i.v. infusion of 3% NaCl solution, 10 ml/kg b.wt., was then given during 45 min. Urine was collected at 15-min intervals during and for 30 min after the end of the infusion. Volume and osmolality of the specimens were measured. Thirty min after the end of the infusion 1 μg DDAVP was given intravenously. After two more 15-min periods urine was collected at 30–60-min intervals for the next 8–10 h.

Water deprivation. The water deprivation test described by Miller et al. (14) was applied. This test was performed months after testing with hypertonic saline infusion. During the time between the tests the patients had been treated with desmopressin (DDAVP) intranasally twice a day. This treatment was withdrawn 24–36 h before the start of the test.

After determination of plasma osmolality the patients were deprived of all fluid, starting at 10 p.m. on the evening before the test. Two patients with severe polyuria were dehydrated from 6 a.m. on the morning of the test. Beginning at 8 or 9 a.m. hourly urine specimens were collected and their volumes and osmolalities were determined. When urine osmolality had reached values with less than 30 mosmol/kg H_2O increase in osmolality between two consecutive collection periods, a new blood sample was drawn for determination of plasma osmolality. DDAVP in an i.v. dose of 1 μg was then injected and hourly urine specimens were collected for 6–10 h. In two of the patients, instead of DDAVP 1 μg AVP was given intravenously.

Treatment with DDAVP. Seven of the patients are given long-term treatment with DDAVP intranasally in doses varying from 3 to 20 μg twice a day (Table I). At home the patients measured their daily urine output for repeated 3-day periods and their DDAVP dosage was adjusted according to the values obtained.

DDAVP and AVP were supplied as investigational preparations by Ferring AB, Malmo, Sweden. DDAVP has a specific and long-lasting antidiuretic action and is devoid of the pressor effects of AVP and the vasopressin preparations (8-lysine vasopressin, pitressin) commonly used (2, 28).

Urine and plasma osmolality were determined with an osmometer (Advanced Instruments Inc.).

RESULTS

Pedigree. It can be seen in Fig. 1 that the disease is transmitted through four consecutive

Table I. *Daily urine volumes before, during and immediately after withdrawal of therapy with DDAVP intranasally according to measurements performed by the patients at home (means of determinations on 3 consecutive days)*

Pat. no.	Sex	Age (y)	Before treatment (l/24 h)	DDAVP dose (μg)	During treatment (l/24 h)	Treatment withdrawn (l/24 h)
1	♂	38	15.2	20 × 2	2.2	21.2
2	♂	42	7.4	10 × 2	2.1	11.0
3	♂	44	6.6	20 × 2	2.0	16.2
4	♀	40	6.6	5 × 2	1.9	10.5
5	♂	56	5.0	10 × 2	1.9	8.1
6	♀	22	3.5	5 × 2	1.7	5.6
7	♀	72	3.5	No treatment		
8	♀	6	3.0	3 × 2	0.6	4.5

Table II *Diuresis and urine osmolality during hydration and infusion of hypertonic saline followed by the i.v. administration of DDAVP*

Pat. no.	Hydration (min)			Infusion of 3% NaCl (min)			N treatment (min)			DDAVP (1 µg i.v. at 0 min) (min)											
	30	45	60	0	15	30	45	60	75	0	15	30	60	90	120	180	240	300	360	420	480
Diuresis (ml/min)																					
1		17.3	18.3		19.3	22.6	28.0	24.4	22.6		8.7	—	4.4	3.7	4.5	1.2	5.0	5.6	7.3	7.9	8.1
2		5.1	7.6		11.6	13.7	21.0	15.6	16.9		2.9	3.1	3.7	2.6	2.4	1.2	2.3	2.2	2.7	2.0	1.3
3		15.0	16.7		18.6	—	22.0	19.4	—		4.1	2.3	1.9	0.5	0.7	1.2	1.9	3.3	2.1	1.5	2.3
4		13.7	15.1		19.3	16.1	18.5	14.2	11.5		6.0	4.9	4.7	1.5	1.5	0.7	1.8	2.4	1.7	1.3	1.2
5		4.5	5.0		8.0	10.1	7.7	4.5	—		[illegible]	1.7	2.0	1.1	2.1	0.8	1.4	2.4	1.3	0.8	0.9
6		12.7	9.3		17.3	9.3	17.4	8.3	9.0		6.0	2.7	2.8	1.5	1.5	2.6	2.4	1.8	1.8	1.5	2.2
7		4.9	5.0		9.9	11.9	14.4	12.5	8.4		5.9	5.3	4.1	3.3	3.0	3.3	1.9	2.2	0.8	[illegible]	2.0
Urine osmolality (mosmol/kg H O)																					
1		40	40		40	50	55	50	50		110	—	225	270	350	310	290	215	190	180	185
2		50	55		45	50	110	165	130		150	410	595	600	610	640	610	620	620	790	700
3		67	63		81	—	88	130	—		175	344	374	438	455	425	385	320	355	380	370
4		104	85		90	110	160	200	200		225	400	465	450	450	480	420	400	490	493	520
5		160	170		125	220	210	225	—		320	275	410	595	600	590	640	630	620	700	700
6		60	90		120	315	190	230	210		300	430	530	510	510	510	550	540	575	650	570
7		200	170		170	195	240	285	305		360	460	480	505	510	505	530	565	580	590	570

generations, that male-to-male transmission has occurred, and that affected males have produced both affected and non-affected daughters. On the basis of these findings a sex-linked recessive inheritance can be ruled out, but the data are consistent with the presence of an autosomal dominant gene.

Hypertonic saline infusion. Table II gives the results of the Carter Robbins test. None of the patients responded with any appreciable antidiuresis during or after the infusion of the saline solution. The urine osmolality increased in most of the patients, but only in patients 6 and 7 were values exceeding the plasma osmolality attained. Thirty min after the administration of DDAVP there was a marked reduction in diuresis and an increase in urine osmolality in all the patients. The long duration of action of DDAVP is well illustrated in Table II. As can be seen, some of the patients did not reach maximum urine osmolality until 7–8 h after the DDAVP injection. The effect lasted for more than 10 h in six of them.

Water deprivation. Table III shows the results of the water deprivation tests. Before the water deprivation the spontaneous diuresis/min was conspicuously high, illustrating the finding that the urine production of the patients increased when treatment with DDAVP was stopped (see below). Table IV gives the plasma osmolality of the patients, determined before and at the end of the water deprivation test. The mean increase in plasma osmolality was 19 mosmol/kg H_2O. The mean duration of the period of water deprivation was 14 h and the mean weight loss 5.0% of the initial b.wt. (Table IV). Table III shows that 4 of the 7 patients were able to decrease their urine volumes during the late part of the water deprivation period. All the patients increased their urine osmolality but only in two cases (nos. 6 and 7) were values exceeding plasma osmolality reached (Table III). The maximum urine osmolality attained by the patients during water deprivation was seen to be roughly proportional to their daily urine production before treatment with DDAVP (Fig. 2).

A modified water deprivation test was performed in patient 8 (not included in Table III). At the start of water deprivation the patient weighed 16.8 kg. Urine osmolality was 65 mosmol/kg H_2O and diuresis/min 2.6 ml. After 14 h of dehydration her weight had fallen to 15.1 kg. At this time the urine osmolality reached a peak value of 405 mosmol/kg H_2O and the diuresis/min had decreased to 0.4 ml. I.v. DDAVP was not given to this patient and plasma osmolality was not determined.

Administration of DDAVP at the end of the

Table III. *Diuresis and urine osmolality before and during the late part of water deprivation, followed by the i.v. administration of DDAVP and AVP*

Pat. no.	Before water deprivation	Late part of water deprivation −3	−2	−1	0	DDAVP (1 μg i.v. at 0 h) 1	2	3	4	5	6 hours
Diuresis (ml/min)											
1	15.0	13.0	14.0	21.0		1.2	1.5	2.3	1.2	0.2	1.8
3	13.0	3.3	2.5	4.1		2.2	0.4	0.3	0.8	0.2	0.7
5	4.0	2.3	3.8	3.5		3.0	1.0	1.0	0.9	1.0	0.6
6	8.3	1.5	1.4	0.8		0.6	0.3	0.3	0.2	0.3	0.3
7	1.7	0.5	0.5	0.5		0.9	0.5	0.2	0.4	0.4	0.4
						AVP (1 μg i.v. at 0 h)					
						0.5	1.5	2.5	3.5	4.5	5.5 6
2	7.4	6.0	3.3	6.2		1.0 1.4	1.0 1.0	2.0 4.0	3.0 8.7	14.2 12.0	6.9 4.0
4	6.1	1.0	2.6	2.3		0.6 0.4	0.7 1.2	1.7 4.4	4.2 10.3	9.8 12.5	9.1 11.4
						DDAVP (1 μg i.v. at 0 h)					
						1	2	3	4	5	6
Urine osmolality (mosmol/kg H₂O)											
1	57	68	83	108		142	196	336	603	595	457
3	77	182	187	211		331	402	485	627	673	681
5	105	222	178	147		209	302	449	573	595	647[a]
6	67	282	282	335		451	643	693	722	775	770
7	285	438	430	426		407	522	534	506	520	586
						AVP (1 μg i.v. at 0 h)					
						0.5	1.5	2.5	3.5	4.5	5.5 6
[illegible]	92	175	158	134		208 247	283 239	205 167	135 100	70 72	82 98
4	75	218	210	189		298 452	441 437	247 96	90 61	57 64	67 67

[a] *A maximum value of 740 was obtained 7–8 hours after the administration of DDAVP*

dehydration caused a reduction of urine flow and an increase in urine osmolality (Table III). In the two patients given AVP instead of DDAVP the duration of action of AVP was found to be not more than approximately 3 h and the maximum urine osmolality reached after AVP was lower than that after DDAVP.

Table IV. *Plasma osmolality before and at the end of the water deprivation period, its duration, and the weight loss during the water deprivation*

Pat. no.	Plasma osmolality (mosmol/kg H_2O) Before water deprivation	At the end of water deprivation	Duration of water deprivation (h)	Weight loss (% of initial b.wt.)
1	285	313	8	7.6
2	295	313	14	4.0
3	283	311	17	4.6
4	295	309	14	3.8
5	288	305	13	3.8
6	289	308	14	3.7
7	290	299	18	2.4
8	—	—	16	10.0

Treatment with DDAVP. All the patients investigated, except no. 7, were given long-term treatment with DDAVP intranasally for 12–18 months. Table I gives the doses and the results of the treatment on the daily urine volumes. As can be seen, 3–20 μg × 2 of DDAVP reduced the daily urine output to about 2 l or less in all the patients. DDAVP was administered intranasally by means of a graded plastic tube (rhinyle) as a solution containing 100 μg/ml. This mode of administration offered no problem to any of the patients. No side-effects were observed during DDAVP treatment and the patients were very satisfied with the therapy.

It was observed that, when the patients stopped



Fig 2. Relation between the daily urine production before treatment and the maximum urine osmolality reached during the water deprivation test.

taking DDAVP their urine production increased markedly compared with the values recorded before the treatment (Table I). Not until 5–7 days after the withdrawal of DDAVP did the daily urine volumes reach the pretreatment level.

Additional laboratory investigations. All the patients investigated had normal values of Hb concentration, haematocrit, WBC, differential count, and serum concentrations of sodium, potassium, calcium, and creatinine. Creatinine clearances were within normal limits, as were the morning levels of plasma cortisol concentration. All patients showed a normal response to ACTH. Protein-bound iodine and T_3-tests were normal in all cases, as were determinations of 17-keto and 17-hydroxy steroids. X-ray examination of femurs and humeri, with respect to fluorosis, revealed nothing abnormal.

DISCUSSION

Hereditary hypothalamic diabetes insipidus is uncommon. Despite this, familial occurrence of diabetes insipidus has long been known (3 13 22). The present patients show the characteristic clinical manifestations of the disease: polyuria and polydipsia starting in early childhood and reaching a peak in adolescence or young adult life. With increasing age, however the intensity of the symptoms tends to lessen. Another interesting feature was the attitude of the patients towards the condition, they regarded it as a peculiar family habit and not as a disease.

In most families with hereditary hypothalamic diabetes insipidus, the disease is inherited as a simple dominant characteristic (13 20, 22, 23 29). However the inheritance may be sex-linked recessive, as demonstrated by Forssman (14). The mode of inheritance within the present family is consistent with the presence of an autosomal dominant gene.

The cause of hypothalamic diabetes insipidus is a total or relative lack of circulating antidiuretic hormone. Whether the primary form of the disease is due to an insufficient synthesis of vasopressin or to a defective mechanism of release through failure of the osmoreceptors has been a matter of dispute (8, 12, 21 22). Martin (22), investigating patients with hereditary hypothalamic diabetes insipidus, found that none of 9 patients tested responded with antidiuresis to infusion of hypertonic saline. However in 3 patients, tested intravenously with nicotine, a definite antidiuretic response was obtained. Nicotine is supposed to act directly on the vasopressin-producing cells and not through the osmoreceptors (15). Because of this, Martin interpreted his results in favour of a failure of the osmoreceptor mechanism rather than a defect in hypothalamic production of vasopressin as a cause of the disease.

The present results showed that none of the 7 patients tested responded to infusion of hypertonic saline with any appreciable antidiuresis, but the urine osmolality increased and in two cases exceeded the plasma osmolality However during water deprivation the diuresis decreased markedly in 5 of 8 patients. This can, to some extent, be explained by a reduction in plasma volume, and also by dehydration being a stronger stimulus for vasopressin release. The urine osmolality increased in all cases, but values greater than plasma osmolality were reached only in 3 These findings suggest that at least 3 patients were able to release vasopressin in response to dehydration and that, according to the criteria proposed by

Miller et al. (24), they can be regarded as having partial diabetes insipidus. As it is reasonable to presume that the pathogenesis of the disease is the same in all affected members of the family the present findings are consonant with the idea that the patients have a limited capacity to produce vasopressin, varying in extent from patient to patient, and that they have a certain ability to release the hormone in response to an increase in plasma osmolality

There are few reports in the literature concerning the pathology of hereditary hypothalamic diabetes insipidus. Generally a marked reduction of the number of cells in nn. supraopticus and paraventricularis, the sites of vasopressin production, has been demonstrated (3 4 16, 17 18). The same findings have been reported in other non-hereditary cases of primary diabetes insipidus (3 7 17) These investigations support the view that a defect in the hypothalamic production of vasopressin causes the symptoms of hereditary hypothalamic diabetes insipidus rather than a selective failure of the osmoreceptors. If it is accepted that the extent of the defect of vasopressin ...tion varies from patient to patient, indi... variation in the severity of the clinical ... can be understood. With such a background of the disease the results of the present investigation, as well as the findings of Martin (22), can be well explained.

In confirmation of the findings in previously reported cases of hereditary hypothalamic diabetes insipidus, the lack of vasopressin in the present patients was found to be an isolated defect. No signs of failing function could be demonstrated in the thyroid gland, the adrenal cortex, or the gonads. Several of the female patients have undergone normal deliveries, and this suggests that their production of oxytocin is adequate.

It is well known that the ability of the kidneys to concentrate the urine is reduced in conditions of prolonged water diuresis (1 5 11). In rats with hereditary hypothalamic diabetes insipidus, this has been shown to be due to a reduction of the renal medullary osmolality through a wash-out effect (19). The ability to concentrate the urine can be improved or normalized by prolonged treatment with vasopressin (5 19). As the present patients had not previously been treated with vasopressin, it was of particular interest to study their renal concentration capacity after administration of the hormone. When given DDAVP in connection with the Carter-Robbins test, all the patients investigated were able to increase their urine osmolality (by 93–600 %), but not to values regarded as normal after vasopressin administration (24).

These findings show that, despite their large production of urine of many years standing, the patients had retained their ability to respond to vasopressin. The two patients who were given AVP instead of DDAVP after water deprivation revealed that DDAVP produced a higher maximum of urine osmolality than did AVP This difference can possibly be ascribed to the higher antidiuretic potency and, above all, to the longer duration of action of DDAVP

That some patients with hereditary hypothalamic diabetes insipidus show a marked decrease in their urine production during later age has been pointed out by several authors (13, 22) Despite the diminishing volumes the urine is still dilute (13). Several of the present patients reported that they had a much larger urine production at the age of 15–25 than later in life. This was particularly conspicuous in patient 7 During water deprivation she reached a urine osmolality of 438 mosmol/kg H_2O and was only able to increase it slightly (by 33 %) after administration of vasopressin. These findings suggest a partial lack of vasopressin. Possibly a combination of partial diabetes insipidus and an age-dependent decrease of the glomerular filtration (10) accounts for the spontaneous decrease of polyuria sometimes seen in patients with hereditary hypothalamic diabetes insipidus during the later part of life.

Most patients with hereditary hypothalamic diabetes insipidus manage well without treatment, and in many instances therapy may not be indicated. When offering substitution therapy to such patients it should be demanded that the preparation given is effective, long-acting, and free from side-effects. These requirements seem to be met by DDAVP (2) but not by other vasopressin preparations available at present.

Seven of the 8 patients investigated were treated with DDAVP intranasally 3–20 μg × 2, for 12–18 months. In all cases this dosage was sufficient to produce daily urine volumes of about 2 l or less. There were no side-effects of the preparation and no difficulty with its administration.

Immediately after a period of treatment with DDAVP the patients were found to produce larger volumes of urine when they stopped taking the preparation than they had produced before starting the therapy. After 5–7 days the urine production returned to pretreatment levels. This is an interesting finding. It might be explained on the assumption that the patients, before the beginning of therapy, had a slight reduction of their body water. The concomitant reduction in plasma volume would then tend to diminish the polyuria. After prolonged DDAVP treatment it is likely that their body water and also plasma volume had increased and thus tended to augment the polyuria immediately on stopping of the drug. A contributory factor, however, might be inhibition of endogenous vasopressin production by DDAVP.

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DIURNAL VARIATIONS IN THE CONCENTRATIONS OF BLOOD ACETOACETATE AND 3-HYDROXYBUTYRATE

The Ketone Body Peak around Midnight and its Relationship to Free Fatty Acids, Glycerol Insulin, Growth Hormone and Glucose in Serum and Plasma

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Abstract In six normal persons reduced physical activity during the day did not influence the rise in blood ketone bodies during the evening, which was previously found in normal persons with normal physical activity. This rise, therefore, does not seem to be due to post exercise ketosis. During the evening free fatty acids (FFA) and glycerol in serum aried in principle in the same ay as did the blood ketone bodies. The rise in blood ketone bodies can therefore be assumed to be due to an increased rate of formation of ketone bodies as consequence of increased mobilization of FFA from adipose tissue. There was no evidence of rise in serum growth hormone as trigger mechanism for the rise in blood ketone bodies. The rise in ketone body concentration was completely prevented by constant glucose infusion.

In a previous study (13) we found characteristic diurnal variations in the blood concentration of acetoacetate (AA) and 3-hydroxybutyrate (3-HB) in normal persons on an ordinary diet and with normal exercise. Particularly marked was a rise in ketone body concentration during the evening to a peak around midnight. Postprandially there appeared to be a reversed relationship between blood glucose and blood ketone bodies.

The purpose of the present investigation was to study 1) whether the rise in ketone body concentration during the evening was due to post exercise ketosis (1), 2) whether it was a consequence of an increased mobilization of fat, 3) whether the increase in blood ketone bodies was preceded by an increase in the concentration of growth hormone and thus possibly related to this and 4) whether a constant infusion of glucose could prevent the rise in ketone body concentration.

MATERIAL AND METHODS

Six healthy students, one woman and five men, aged 22 28 years (mean 4), were studied. None of the subjects are overweight and none in regular athletic training. They arrived at the hospital at 8 a.m were immediately put to bed and remained in bed until the conclusion of the study at 1 a.m. on the next day. They had breakfast at 8 a.m. (56 g carbohydrate, 28 g protein, 25 g lipid), lunch at 12 noon (104 g carbohydrate, 23 g protein, 17 g lipid) and dinner at 5 p.m. (52 g carbohydrate, 77 g protein, 21 g lipid), making total of about 1 800 kcal. Only water was given between meals, and the subjects wer not permitted to smoke.

An indwelling catheter was inserted into an antecubital vein at 5 p.m., and from 6 p.m. blood samples (15 ml) were removed from this catheter every half hour. Saline (0.9%), 1–2 ml, was used occasionally to keep the catheter patent. Blood samples for determination of free fatty acids (FFA), glycerol, insulin and growth hormone were centrifuged after coagulation, serum was removed by pipette and stored at −20°C until analysis the next day. Blood for determination of AA, 3-HB and glucose was collected in tube containing EDTA as anticoagulant. Until analysis on the following day blood for ketone body determination as stored in buffered state at −20°C (12), hile blood for glucose determination was centrifuged, the plasma removed and stored at −4°C.

Approximately one month later the study was repeated This time continuous i glucose infusion (5.5%) was given from 6.30 p.m. to 1 a.m. otherwise the schedule was identical to the first experimental period. The amount of glucose given corresponded to caloric amount of 120% of the basal caloric requirement. Blood ketone bodies were determined by an enzymatic micromethod (12), plasma glucose was measured by an o-toluidine method (7). FFA in serum were analysed by colorimetric method, based on the formation of FFA-Cu soaps, using palmitic acid as standard (3). Serum glycerol was determined by an enzymatic procedure (4). Boehringer kits (Boehringer/Mannheim, West Ger

many) were used in the determination of both FFA and glycerol. Serum insulin and serum growth hormone were measured by a radioimmunoassay technique employing wick chromatography (15, 16).

RESULTS

AA, 3-HB, FFA, glycerol, insulin and glucose

The concentration of AA and 3-HB in blood, FFA, glycerol and insulin in serum and glucose in plasma varied in a characteristic way in all 6 individuals studied in each of the two experimental situations. Fig. 1 gives the mean concentration ±S.E.M. of the above mentioned substances at the various times of sampling in the studies without and with continuous glucose infusion.

For each substance variance homogeneity was confirmed using Bartlett's test at each sampling time. With Fisher's test for linear regression it was established that the mean concentrations after a given time might follow a linear course. If the slopes of these lines differed significantly from zero, using Student's *t*-test, a significant ...n mean concentrations was proved.

In the studies without glucose infusion (Fig. ...A) it can be seen that the concentration of both AA and 3-HB began to increase from 9.30 p.m., after a transient postprandial decrease. The increase in mean concentrations from 9.30 p.m. was statistically significant ($p < 0.001$). The highest mean concentrations of AA and 3-HB were 108 μmol/l (range 87–205) and 144 μmol/l (range 86–264). In principle the concentrations of FFA and glycerol in serum changed in the same way. The concentrations fell postprandially and then increased significantly, FFA from 8.30 p.m. to 1 a.m. ($p < 0.001$), glycerol from 8.30 to 11.30 p.m. ($p < 0.005$). Thus the concentrations of these two substances began to increase before the concentration of the ketone bodies. After the transient postprandial increase in plasma glucose and serum insulin during the beginning of the evening, there were only small fluctuations in concentrations during the rest of the study period.

In the studies employing continuous glucose infusion (Fig. 1 B) there was a slight but statistically significant fall in the blood concentrations of AA ($p < 0.01$) and 3-HB ($p < 0.001$) from 6 p.m. to the end of the study period. FFA and glycerol did not change significantly after an initial postprandial fall. After a transient rise in plasma glucose and serum insulin in association with the initiation of the glucose infusion, the concentration of these substances fell to a level which was clearly higher than in the studies without glucose infusion.

Growth hormone

The mean concentration ±S.E.M. of serum growth hormone at the various times of sampling in each of the two experimental situations is given in Fig. 1. In the studies where glucose infusion was not used (Fig. 1 A) a slight rise in the mean concentration of serum growth hormone was seen at 8 p.m., caused by a rise in concentration in two of the subjects. The most characteristic finding was, however, a significant increase in serum growth hormone late in the evening in all subjects studied, the mean concentration beginning to rise from 9.30 p.m. to a peak at 11 p.m. Thus the rise in the mean concentration of serum growth hormone occurred at the same time as the rise in the mean concentrations of AA and 3-HB in blood. However, only in one subject did the rise in serum growth hormone occur at the same time as the rise in blood ketone bodies; in the other five subjects the rise in serum growth hormone was preceded by the rise in blood ketone bodies.

In the studies employing glucose infusion (Fig. 1 B) a significant rise in serum growth hormone in the evening occurred in all subjects studied, the mean concentration reaching a peak at 11 p.m.

DISCUSSION

In the studies in which glucose infusion was not used, the concentration of both AA and 3-HB increased during the evening in all persons studied. The highest concentrations of AA and 3-HB were 108 ± 35 μmol/l (mean ±S.D.) and 144 ± 71 μmol/l (mean ±S.D.), respectively. These concentrations are of the same order of magnitude as those seen in normal, non-obese persons given the same diet but with normal physical activity, the midnight peak concentrations here being 113 ± 43 μmol/l (mean ±S.D.) for AA and 153 ± 88 μmol/l (mean ±S.D.) for 3-HB (13). The reduction in physical activity employed in the present investigation did not, therefore, in-

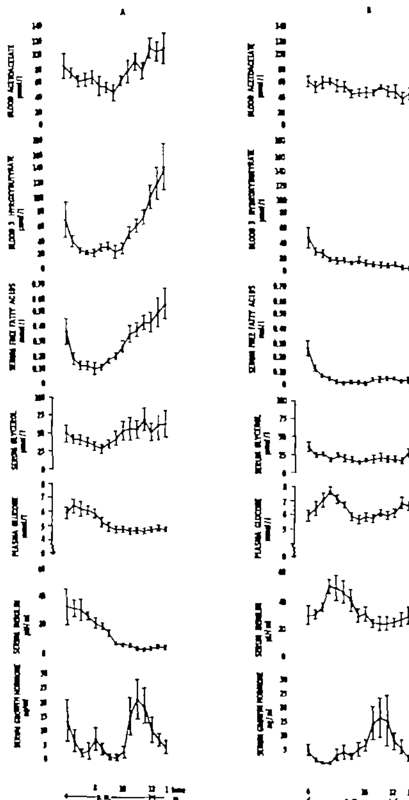


Fig. 1. Blood AA, blood 3-HB, serum FFA, serum glycerol, plasma glucose, serum insulin and serum growth hormone (mean ± S.E.M.) at various times during studies without (*A*) and with glucose infusion (*B*).

fluence the physiological rise in ketone body concentration in blood during the evening. Consequently this rise cannot be explained on the basis of post-exercise ketosis.

The metabolic changes which accompanied the rise in ketone body concentration were in agreement with this conclusion, as serum glycerol increased in parallel with the ketone body concentration in blood. By contrast serum glycerol falls with post-exercise ketosis in the period after exercise (9 10) Since serum glycerol is an indicator of mobilization of adipose tissue FFA, the present finding of a rise in glycerol and FFA in serum preceding the rise in ketone bodies in blood is indicative of an increased mobilization of FFA from adipose tissue. An increasing concentration of FFA in serum usually causes an increased rate of formation of ketone bodies. Thus the rise in ketone body concentration during the evening may be assumed to be due to an increase in the rate of formation of ketone bodies.

The trigger mechanism for the rise in serum FFA might be the preceding fall in serum insulin. This is supported by the results in the glu infusion experiment, in which the insulin is were maintained elevated and FFA did not rise.

There is experimental evidence that growth hormone stimulates ketogenesis under certain conditions (2, 5 6, 8). Since the concentration of serum growth hormone increases 4–6 hours after ingestion of glucose (11 14) one might expect a close temporal relationship between the increases in blood ketone bodies and serum growth hormone in the evening. In the studies without glucose infusion the rise in the mean concentrations of blood ketone bodies and serum growth hormone occurred at the same time, but in no case did the rise in serum growth hormone precede the rise in blood ketone bodies. Thus serum growth hormone cannot be the trigger mechanism for the increase in blood ketone bodies. In agreement with this is the observation that the increase in serum growth hormone during the evening was not accompanied by an increase in blood ketone bodies in the experiment where glucose infusion was employed.

The concentration of ketone bodies fell in association with the postprandial hyperglycaemia and rose only after the plasma glucose had again fallen to normal basal concentrations. On continuous glucose infusion, when plasma glucose and serum insulin were at a higher level than in the study not employing glucose infusion, the rise in blood ketone bodies during the course of the evening was completely prevented. The previously found inverse relationship between plasma glucose and blood ketone bodies within the normal range of concentrations of these substances (13) was thus confirmed in the present investigation.

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DEXTROSTIX REFLECTANCE METER AS AN AID IN DIAGNOSTIC HYPOGLYCEMIA

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Abstract. The Dextrostix Reflectance Meter (DRM) system has been used for monitoring of the blood glucose level during diagnostic hypoglycemia in 16 patients with suspected pituitary insufficiency In the range 15–40 mg glucose/100 ml blood the reproducibility of the DRM system, ± 1.7 mg/100 ml, was as good as that of the reference method. The agreement between values obtained with the DRM and the reference method showed straight line relationship, the equation for the regression line being $y = 1.02x - 3.1$ and the coefficient of correlation 0.94. The access to immediate and continuous information about the blood glucose level as of value for avoidance of complications and methodological misinterpretations, and permitted the test to be performed in open care.

The rise of plasma cortisol and growth hormone following an insulin-induced hypoglycemia is preferred by many as a test of pituitary reserve capacity (3 4). However in subjects with low or absent pituitary reserve capacity the blood glucose level may fall to dangerously low levels after i.v. administration of insulin. In other cases the hypoglycemia may not be pronounced enough to provoke the pituitary to increased activity Such factors could be better controlled if immediate and continuous information about the blood glucose level during the test were obtainable. The modern and automated central chemical laboratory as a rule cannot meet such requirements. The only method at present offering that rapid information about the blood glucose is the Dextrostix Reflectance Meter system (DRM) (Ames Co. Elkhart, Indiana, USA). In this system the colour developed on the glucose oxidase test strip is read with a standard light source (Reflectance Meter), thus minimizing the observer error inherent in ocular reading of the colour Several authors have studied this method in the normal and hyperglycemic range (1 2, 5 6, 8) but little is known about its accuracy in hypoglycemia. In the present study we report our experiences with the DRM in diagnostic, insulin-induced hypoglycemia, with special regard to its performance at low blood glucose levels.

PATIENTS AND METHODS

The subjects studied included 5 normals, 6 acromegalics, and 5 patients with chromophobe pituitary adenoma. All subjects had fasted overnight and were kept supine from 30 min before and throughout the test period.

After the control period of 30 min crystalline insulin, 0.1 IU/kg b.wt., was given intravenously With an indwelling plastic catheter blood samples were drawn into syringe every 10–15 min from −10 to 90 min. Directly from the syringe 0.1 ml blood was taken by pipette and added to 4 ml of precipitant ($ZnSO_4$–NaOH) chilled to 4°C in ice cubes for later whole blood glucose determination according to *Marks* (7). Simultaneously large drop of blood was transferred from the syringe to the Dextrostix strip, care being taken to cover its reagent field completely This process took about 3 sec. Timing as then commenced and at the end of exactly 60 sec the blood was washed off with jet of water the strip blotted slightly and the blood glucose value read immediately from the Reflectance Meter This process was repeated with another strip with blood from the same syringe to obtain duplicate determinations.

T eliminate the risk of differences in sensitivity between batches of Dextrostix and of misleading results from deteriorated Dextrostix strips serum standard (Autonorm®) as used as control. The serum standard was processed in the same way as blood in the DRM system.

RESULTS

The random error of the DRM method within the blood glucose range 15–85 mg/100 ml was

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THE EFFECT OF BENDROFLUMETHIAZIDE ON THE INTESTINAL ABSORPTION OF CALCIUM IN NORMOCALCAEMIC RENAL STONE FORMERS AND IN HYPERPARATHYROIDISM

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Abstract. While the hypocalciuric action of thiazides is well established, their action on the intestinal absorption of calcium has remained matter of dispute. In the present investigation this action has been studied in man by the measurement of forearm radioactivity in an Armac large volume liquid scintillation counter following separate oral and i. administration of ^{47}Ca. Determined in this way duplicate measurements of calcium absorption in the same individual yield standard deviation of 3.2%. Oral administration of bendroflumethiazide, 10 mg/day led to significant increase in fractional calcium absorption in group comprising patients ith hypercalcaemic hyperparathyroidism ($n=7$), normocalcaemic renal stone disease ($n=8$) and postsurgical hypoparathyroidism ($n=1$). The mode of action of thiazides on the intestinal calcium absorption is discussed. Probably thiazides promote in specific way the transport of calcium through the intestinal as well as through the tubular epithelial cells.

A reduction in renal excretion of calcium during thiazide administration is an established phenomenon in normocalcaemic and hypercalcaemic patients (13, 17, 32) and in experimental animals (10). The effect of diuretic thiazides on the intestinal calcium absorption has not been clarified.

Measurements of the *net* absorption of calcium by balance technique yield controversial results. Nassim and Higgins (22) observed a decreased net absorption in two, but no change in five patients. Similarly the net absorption remained unchanged in nine studies by Harrison and Rose (7), while on the other hand, Yendt et al. (32) found that thiazide increased the net absorption in six of seven studies.

Conflicting results are also obtained by the application of isotope techniques. As judged from the determination of peak radioactivity following a single oral dose of Ca, thiazides decrease calcium absorption in nine of ten patients (6). Conversely with a more elaborated and reliable technique combining an i.v. injection of ^{45}Ca with orally administered ^{48}Ca, Lekkerkerker et al. (18, 19) demonstrated an increased intestinal absorption of calcium during thiazide administration in eight hyperparathyroid and nine normocalcaemic patients but no change in eight hypoparathyroid. Consequently further studies are needed to elucidate this problem.

The present investigation applies the method of Lutwak and Shapiro (20) using ^{47}Ca and counting by liquid scintillation, to the problem and is a part of a comprehensive survey of the effect of diuretic thiazides on calcium metabolism (10, 11, 12, 13, 14).

MATERIAL AND METHODS

Seven hyperparathyroid patients (nos. 10–16), eight normocalcaemic renal stone formers (nos. 9) and one postsurgical hypoparathyroid patient (no. 1) substituted with vitamin D and thyroxine are investigated before and during thiazide administration.

Except for patient 15 the hyperparathyroid patients were all hypercalcaemic as judged from serum total and/or ionized calcium determinations, and had values of TRCa%, within the range of hyperparathyroid patients studied on similar diet (30). Patient 15 was operated upon because of high concentration of total serum calcium prior to the present investigation, and parathyroid adenoma as found. The normocalcaemic renal stone formers had at routine determinations serum total calcium values within the upper part of the normal range, but the finding of normal levels of serum ionized calcium in all rendered the diagnosis of hyperparathyroidism unlikely. The age, sex and average serum concentrations

Table I. *Fractional intestinal calcium absorption before and during bendroflumethiazide (TZ) administration (10 mg/24 h) and serum calcium values of the patients investigated*

Pat. no.	Age (yr)	Sex	Serum concentration (mEq/l) TOCa	UFCa	Ca^{++}	Fractional intestinal calcium absorption (%)	
			Control range (mean ± 2.56 S.D.)				
			4.56–5.28	2.75–3.25	1.91–2.47	Before TZ	During TZ
1	30		4.63	2.64	1.75	56.6	70.4
2	45	♂	5.01	3.12	2.13	48.3	57.1
3	56	♂	5.10	3.29	2.47	27.5	34.5
4	54	♂	4.78	2.98	2.77	52.5	54.2
5	50	♂	5.02	2.84	2.13	30.6	37.1
6	51		5.11	3.02	2.30	39.5	51.6
7	58		5.45	3.13	2.22	59.8	49.6
8	17		5.17	3.15	2.37	51.5	58.2
9	43		5.01	3.08	2.44	28.3	39.8
10	39	♂	5.88	3.78	2.69	39.4	36.3
11	24		5.60	3.69	2.68	58.9	61.7
1	55		5.74	3.56	2.67	47.0	48.2
13	60		5.79	3.55	2.71	51.8	55.7
14	66	♂	5.28	3.35	2.59	51.5	61.7
15	57		5.26	—	—	50.8	74.0
16	61		5.80	—	—	56.5	73.9

of total, ultrafiltrable and ionized calcium are presented in Table I.

For the study of intestinal calcium absorption the technique of Lerwak and Shapiro (20) as modified by Wills et al. (31) was used. All patients underwent a 10-day metabolic study taking a standard diet composed of approximately 800 mg calcium, 1 000 mg phosphorus, 70–100 mEq sodium and 1 g protein/kg b.wt./day.

Bendroflumethiazide, 10 mg/24 hours, was administered during the last 5 days of the period. On day 2 all patients were given 3 µCi ^{47}Ca as $CaCl_2$ (Amersham, England) intravenously and 4 hours later the opposite arm was counted for 10 min in a large-volume liquid scintillation counter (Armac, Packard Instruments). The total number of counts was assumed to be equivalent to the forearm retention of tracer which would result from an intestinal absorption of 100%. On days 5 and 10 an oral dose of 4 µCi ^{47}Ca was taken by the fasting patient in 50 ml distilled water with 200 mg ^{40}Ca ($CaCl_2$, $2H_2O$) as carrier. The forearm was counted for 10 min before and 4 hours after the oral dose. The ratio between the forearm count after the i.v. dose and after the oral dose corrected for decay and differences in doses of ^{47}Ca administered was taken to represent the fractional calcium absorption.

To ascertain the reproducibility of the method within a 10-day metabolic period, nine normocalcaemic patients without any sign of calcium metabolic disturbances were investigated without thiazide administration (Table II).

RESULTS

The results of the fractional intestinal calcium absorption before and during thiazide administration are presented in Table I. In all sixteen patients except two (nos. 7 and 10) the intestinal absorption of calcium increased during thiazide administration. This increase is statistically significant when the hyperparathyroid and the normocalcaemic patients are considered together ($p < 1\%$, Wilcoxon's test of paired differences).

The results of duplicate determinations of intestinal calcium absorption in normocalcaemic patients are presented in Table II. The results show no difference between the two determinations ($p > 10\%$, Wilcoxon's test of paired differences), and the standard deviation of the duplicate determinations in the same patient is 3.–%.

DISCUSSION

Since the calcium balance technique is less accurate than most isotope techniques and measures *net* instead of true absorption, isotope techniques are more suitable for studies of the intestinal calcium absorption. According to Heaney (9) the most reliable methods are the various modifications of the double isotope technique and of the technique involving separate oral and i.v. administration of a single isotope. The present technique, which belongs to the latter group, presupposes steady state conditions during the period

Table II. *Reproducibility of the fractional intestinal absorption of calcium determined after 5-day intervals in 9 patients with arteriosclerotic heart disease*

Pat. no.	Age (y.)	Sex	Fractional intestinal calcium absorption ( ) Day 5	Day 10
1	57	♀	23.0	28.8
2	49	♂	38.8	46.8
3	74	♂	28.9	27.1
4	59	♂	45.1	38.8
5	58	♂	36.6	35.1
6	71	♂	38.1	33.1
7	47	♂	26.8	28.7
8	57	♂	32.1	31.2
9	56	♂	35.0	38.0

f investigation. In principle, however this is not fulfilled with the present schedule, involving a reduction of about 50% in the renal calcium excretion (13), but the error introduced in this way is minimal. The increased retention of tracer in consequence of thiazide administration may be estimated to represent less than 1% of the total number of counts accumulated in the forearm following the i.v. dose. Such a change will not alter the final figure of fractional calcium absorption by more than 1%. Since ^{47}Ca equilibrates with about 2 000 mg of the miscible calcium pool within a few hours (8) it may be estimated that a calcium retention of 25 mg/4 h during bendroflumethiazide administration (13) (corresponding to a 50% retention in a subject having a calciuresis of 300 mg/24 h before thiazide administration) may cause an expansion of about 1% of the fast component of the miscible calcium pool. Thus, within the 4-hour period elapsing between i.v. ^{47}Ca administration and forearm counting, bendroflumethiazide may cause a 1% increase in the trapping of ^{47}Ca within the forearm. Calculations reveal that such a change will affect the final figure of fractional calcium absorption by 1% or less, this difference being well within the error of duplicate determinations (Table II).

Consequently our finding of an increased intestinal absorption of calcium during thiazide administration remains valid. This finding also agrees with the results of similar studies carried out by comparable techniques (2, 18). No explanation can be offered for the contradictory results of Gursel (6) obtained through the determination of peak plasma values of ^{47}Ca following oral administration of the tracer.

The mechanism through which the enhancement of calcium absorption in the intestines takes place is unknown. Since thiazide administration either does not affect the secretion of parathyroid hormone (28) or even depresses its secretion (3), other mechanisms have to be looked for. Metabolic alkalosis leads to an increased intestinal absorption of calcium (29) and the metabolic alkalosis of thiazide administration might therefore cause the increased absorption.

The similarities between the low resistance epithelia of the intestine and the kidney in anatomy (1 21 23 24) and transport mechanisms (4 25 26, 27) suggest that the mechanism of the increased calcium absorption during thiazide administration is the same in both sites. A metabolic alkalosis is also seen during furosemide and ethacrynic acid administration (15) without any reduction in renal calcium excretion. The difference in renal handling of calcium in spite of the same metabolic alkalosis might mean that the alkalosis is without any importance for the calcium retention during thiazide administration. Another possibility is that the increased alkalosis during furosemide and ethacrynic acid administration is compensated by a calciuretic effect of these diuretics due to other sites of action (16).

Consequently the question whether the increase in intestinal and tubular calcium absorption during thiazide administration is due to a specific effect on the kidney and intestine or is secondary to a metabolic alkalosis or a combination of these two possibilities remains to be solved. Likewise the consequence of the calcium retention during thiazide administration is unknown and needs further attention.

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INHIBITORY EFFECT OF L DOPA ON COLLAGEN BIOSYNTHESIS

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Abstract. The effect of L-dopa on connective tissue biosynthesis by 10-day-old chicken embryos has been studied. Inhibited collagen formation as expressed by re duced hydroxylation of (^{14}C)proline and (^{14}C)lysine, and similarly reduced glycosylation of (^{14}C)hydroxylysine as found. No influence on the biosynthesis of glyco- and/or mucoproteins was observed.

In a previous paper the authors reported on the effect of L-tyrosine and L-phenylalanine on collagen biosynthesis (2). It was demonstrated that L-tyrosine inhibits the incorporation of (^{14}C)proline (Pr) into proteins and the biosynthesis of (^{14}C)hydroxyproline (Hypro). L-phenylalanine af fected neither the incorporation of (^{14}C)Pr and (^{1} C)lysine (Lys) nor their hydroxylation to (^{14}C)-Hypro and (^{14}C)hydroxylysine (Hylys) in the undialyzable material synthesized by 10-day-old chicken embryo tibiae. In this communication the effect of the related amino acid L-dopa (dihydroxyphenylalanine) on collagen and glyco- and/or mucoproteins is reported. The parameters considered were the hydroxylation of the amino acids (^{14}C)Pr and (^{14}C)Lys, the glycosylation of (^{14}C)Hylys, and the incorporation of (^{14}C)glucosamine. The extens e use of L-dopa in long-term treatment of Parkinson s disease stimulated this study of its effect on connective tissue.

MATERIAL AND METHODS

Embryonated chicken eggs were purchased from the State Serum Institute of Copenhagen. Uniformly labeled (^{14}C)L-Pr 197 μC/μmole, (^{14}C)L-Lys, 40 μC/μmole, and (^{14}C)D-glucosamine, 5–10 μC/μmole, were obtained from New England Nuclear Corp L-dopa was product of Sankyo, Japan.

Ten-day-old chicken embryo tibiae wer removed by microscopic dissection. The tissues ere preincubated during 1 hour in medium of buffer glucose and inorganic salts (8). Increasing doses of L-dopa ere then added and the preincubation was continued for 30 min. The medium without L-dopa was used for control.

After preincubation 5 μC (^{14}C)Lys or (^{14}C)Pr or (^{14}C)glucosamine ere added for an incubation period of 2 hours. At the end of this period the tissues were homogenized and the homogenates dialyzed against running tapwater overnight. The undialyzable (^{14}C)Pr and (^{14}C)glucosamine labeled materials ere hydrolyzed in 6 N HCl at 110°C for 16 and 4 hours, respectively. The material labeled with (^{14}C)Lys as divided into 2 aliquots. One was hydrolyzed as indicated bove for the (^{14}C)Pr labeled material and used for determination of total (^{14}C)Hylys, while the unhydrolyzed aliquot was used for determination of unglycosylated (^{14}C)Hylys (3). By subtraction the value of glycosylated (^{14}C)Hylys was obtained. Th HCl was eliminated from the acid hydrolyzed samples by evaporation under vacuum t 65°C. The total (^{14}C) incorporated was determined on an aliquot of the (^{14}C)Pr (^{14}C)Lys and (^{14}C)glucosamine labeled material and expressed as percentage of controls. Total and unglycosylated (^{14}C)Hylys were assayed on corresponding hydrolyzed and unhydrolyzed aliquots of the (^{14}C)Lys labeled material according to Blumenkrantz and Prockop (3). (^{14}C)Hypr was assayed by the method of Juva and Prockop (7).

The radioactivity as measured in liquid scintillation counter and the observed cpm were corrected to dpm by the use of internal standards. The results ere calculated as dpm per bone per hour of incubation and expressed as percentage of controls

RESULTS

When tibiae of chick embryos were incubated with (^{14}C)Pr in the presence of increasing concentrations of L-dopa, decreasing amounts of (^{14}C)Hypro were synthesized. Although less pronounced, a reduced incorporation of (^{14}C)Pr was also observed. When, in parallel experiments, tissues were incubated with (^{14}C)Lys in the presence of increasing concentrations of L-dopa, decreasing amounts of (^{14}C)Hylys were synthesized,

Table I *Effect of increasing concentrations of L-dopa on uptake of (14C) Pr and (14C) Lys on their hydroxylation, and on the glycosylation of (14C)Hylys by 10-day-old chicken embryo tibiae*

Results are expressed as % of controls. Values represent average of two separate assays

L-dopa (mM)	(14C) Pr	(14C) Hypro	(14C) Lys	(14C)Hylys Total	Glycosylated
0.85	111	91	105	80	79
1.7	103	81	88	52	45
7	78	25	88	39	39
10	68	26	92	38	38
17	62	23	91	25	25

Table II. *Effect of L-dopa on the uptake of (14C)D-glucosamine by 10-day-old chicken embryo tibiae*

L-dopa (mM)	Total uptake of (14C)-D-glucosamine (% of control)
0.85	89.8
10	81.7
17	94.7

ile the incorporation of the precursor (14C)Lys not inhibited.

Glycosylation of (14C)Hylys was decreased parallel to the hydroxylation of (14C)Lys (Table I). No significant effect of L-dopa on the incorporation of (14C)glucosamine into glyco and/or mucoproteins was observed at the dosages used (Table II).

DISCUSSION

The decreased incorporation of (14C)Pr under the effect of L-dopa may be due to an inhibitory competition for a similar transport system as observed between the related amino acid L-tyrosine and (14C)Pr (2). (14C)Lys is not affected on the basis of its transport by the basic amino acid pathway (6 9). According to Blasberg (1) the transport system that carries L-dopa into brain cells is probably shared with other neutral amino acids including phenylalanine and tyrosine. Our finding is in agreement with the statement of Wurtman et al. (10) that, although exogenous L-dopa is apparently not incorporated into proteins, it may inhibit protein synthesis by sharing the transport system with related amino acids.

The finding that the biosynthesis of both (14C)-Hypro and (14C)Hylys is more vigorously decreased than the incorporation of their precursor amino acids suggests that an underhydroxylated collagen is synthesized in the presence of L-dopa. Considering that some amino acids are known to chelate various metal ions (5), this effect could be due to a chelation of Fe ions, one of the cofactors required for the hydroxylation step.

Our findings of an unaltered incorporation of (14C)D-glucosamine into glyco- and/or mucoproteins synthesized by chicken embryo tibiae under the effect of L-dopa are comparable to the non-affected glycoprotein biosynthesis by rat liver mitochondria observed by Bosman et al. (4).

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RESPIRATORY DISTURBANCE DURING L DOPA TREATMENT OF PARKINSON'S SYNDROME

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Abstract. A 56-year-old woman, suffering from post encephalitic parkinsonism for 15 years, developed re spiratory disturbance in connection with L-dopa therapy. About 2 hours after the medication she often had at tacks of irregular breathing, lasting for 2–4 hours. During the attacks apnoeic periods of 2–30 sec appeared, inter spersed in uniform series of vigorous breaths. Arterial oxygen saturation was never below 90%. The intestinal absorption and the elimination from the blood of L-dopa did not differ from that observed in other patients with parkinsonian symptoms. A single-blind study confirmed the impression from history and clinical observation that the disturbance is caused by L-dopa.

L-dopa treatment of Parkinson's syndrome may cause a number of side-effects, such as nausea, orthostatic or postural arterial hypotension, ar terial hypertension, increased libido menstrual disturbances, hyperkinesia, muscular hypotonia, insomnia, euphoria-hypomania, confusion, hal lucinations and depression (3). We have recently observed a patient with a disturbance of the re spiration in the course of L-dopa treatment. The purpose of the present paper is to describe this respiratory disturbance and its relationship to L dopa treatment. The absorption and turnover of the L-dopa in this patient were also studied and compared to those of other parkinsonian patients.

CASE REPORT

The patient was 56-year-old woman. At the age of 38 she developed encephalitis lethargica. Parkinsonian symptoms appeared about 1 year later. Increased during 1–2 years and then remained relatively stationary. These symptoms are predominantly in the left side of the body and consisted of moderate rigidity hypokinesia and tremor. She had also oculogyric crises. She was right-handed and could therefore manage her activities of daily living (ADL) acceptably. Since the age of 49 she has been treated with anticholinergic drugs (benzhexolchloride 5 mg 3/day) with certain effect. In Dec 1969 when she was 53 years old, L-dopa treatment was started additionally. A maintenance dose of 2.4 g/day divided into four equal doses, had good effect on all symptoms ex cept the oculogyric crises. Before treatment she had a moderate handicap, ADL group 2; during treatment she became free from parkinsonian symptoms most of the day ADL group 0 (3).

About 2 months after the start of L-dopa treatment the patient began to complain of attacks of respiratory dis comfort and increased salivation. These attacks appeared about 2 hours after the intake of L-dopa and lasted for 1–2 hours. They did not occur after each L-dopa dose, but usually at least once day and then after the morning dose. Improvement of the parkinsonian symp toms was generally seen half an hour after the L-dopa intake, thus before the onset of these attacks. The pa tient found the attacks very disturbing. Her general condi tion was, however not more affected than that she dared to undertake, g., railway journeys alone.

A clinical examination revealed that the attacks con sisted of irregular series of vigorous breathing, inter rupted by periods of apnoea. At the same time increased salivation occurred. The patient was not cyanotic, and at auscultation of the lungs no abnormal findings were made. X-ray of the lungs was normal. The patient re vealed no sign of heart disease, but since the age of 51 she had had slightly increased BP systolic usually 160–180 and diastolic 100–110 mmHg. The roentgenological heart volume and ECG were normal. No retinopathy was present and serum creatinine concentration was normal. No haematological abnormalities, disturbances of the liver function or changes in the serum electrolytes wer re vealed by routine laboratory tests. The standard bi carbonate level, on two occasions during periods with respiratory disturbance, was 27 mmol/l, pCO_2 41 and 43 mmHg, and pH 7.41 and 7.43, respectively.

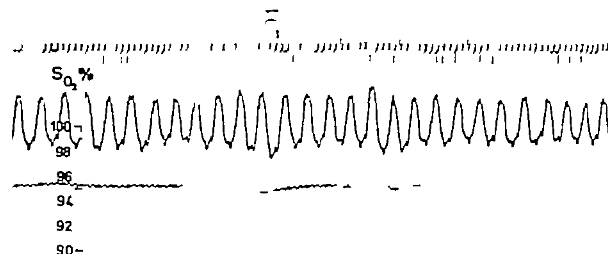


Fig. 1. The patient's breathing before L-dopa medication. *Tracings from above: 1) Time markings at 1 sec intervals.* 2) ECG. 3) Spirogram. Inspiration upwards. 4) Arterial oxygen saturation (ear oximeter), calibration to the left.

METHODS

patient breathing was recorded on a direct writing ograf (Elema, S eden) with the aid of capacitance w transducer placed on band round her thorax. At the same time her arterial oxygen saturation was monitored ith an ear oximeter (Atlas-Werke, Bremen) (5), calibrated ith spectrophotometric saturation determinations on arterial blood from the brachial artery in which the BP was determined directly through small polyethylene tube with the aid of capacitance manometer (Elema).

The absorption of dopa was studied by determination of the radioactivity in urine after i. and oral administration of L-3,4-dihydroxyphenylalanine-1-^{14}C. At the first investigation solution containing 23 µCi of the labelled compound in 1 mg unlabelled L-dopa was injected i. 30 min after the oral administration of a tablet containing 0.5 g L-dopa. At the second investigation 23 µCi of the radioacti compound in 0.5 g inactive L-dopa was given orally in two half tablets of 0.25 g, one of which contained the radioactive compound. The orally administered L-dopa was given together with standardized breakfast consisting of one glass of milk and two slices of bread and butter. The recovery of radioactivity in urine was determined for 3 days after each experiment. The absorption of L-dopa was calculated from the formula

$$A = Uo/Ui$$

where A is the fraction of the L-dopa which was bsorbed (or more correctly the fraction that reached the general circulation) and Uo and Ui are the recoveries in urine after the oral and i.v. administrations of the labelled L-dopa, respectively.

During the study when L-3,4-dihydroxyphenylalanine-1-^{14}C as gi en i. repeated blood samples were taken for determination of the plasma disappearance rate of L-dopa. The total radioactivity of the dopa fraction was determined after ion-exchange chromatographic separation of dopa from other labelled metabolites. The radioactivity was measured ith Packard Tri-Carb liquid scintillation counter. The concentration of unlabelled dopa was measured by an ion-exchange chromatographic method (4). For comparison the bsorption and turnover of L-dopa were studied similarly in another 10 parkinsonian patients, none of whom complained of breathing difficulties. They had been on L-dopa treatment for 1 years. The dose required varied between 1.6 and 6.0 g/day (mean 3.5). The results of the studies in these 10 patients ill be described in detail elsewhere (2).

RESULTS

Respiratory studies

The patient was examined on six occasions. On three occasions L-dopa alone was given. During one examination chlorpromazine was given i.m. after L-dopa administration. On two occasions placebo was given, 16 hours and 3 days after the previous L-dopa dose respectively. During the in-

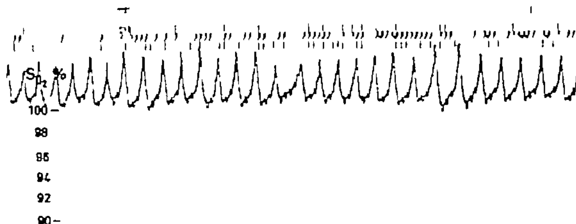


Fig. 2. Breathing type 1 / hours after L-dopa medication.

vestigation she was recumbent, except for about half an hour when she was sitting to have breakfast and to take the L-dopa. During the investigation period the treatment with 5 mg benzohexol-chloride three times a day was continued.

In the morning, when the examination began, the patient usually breathed regularly (Fig. 1). The type of breathing slowly began to change about 1 hour after the L-dopa intake, the breaths becoming somewhat more sudden and short lasting with more marked intervals (Fig. 2). Later brief pauses in the breathing appeared irregularly these periods of apnoea becoming longer and more frequent during about 1 hour (Fig. 3).

The longest apnoea recorded extended over 34 sec; durations of 10–20 sec were seen up to about 40 times per hour. About 4 hours after the L dopa intake these irregularities began to subside, although they did not disappear entirely during the period of recording, which was maximally 6 hours. As seen in Figs. 1–3 oxygen saturation was about normal during the spells of respiration, falling somewhat in the periods of apnoea and for the first seconds of breathing after the pauses, but only by some 2–5%. It was never observed to fall below 90%. Thus there was no serious hypoventilation.

Inhalation of 100% oxygen was given on three occasions. It did not influence the irregularity of the respiration, the arterial oxygen saturation rose and showed only very slight reductions even after long periods of apnoea (Fig. 3).

In order to block the dopamine receptors, during one examination chlorpromazine (Hibernal®)



Fig. 3. Three hours after L-dopa. Lowest tracing arterial BP calibration to the right. The regularly appearing, small deflections in the spirogram are artefacts from the heart beats.

was given in two i.m. injections of 10 mg each, 3 and 3 1/2 hours after the L-dopa medication. It had no influence on the pathological respiratory pattern, but a sedative effect could be observed.

The irregular respiration was observed during the recording period each time L-dopa was given. Six apnoea periods of more than 10 sec duration in about 2 hours could also be observed when placebo was given 16 hours after the previous L-dopa dose. Placebo was also given at an examination when 3 days had elapsed since the last dose of L-dopa and parkinsonian symptoms had reappeared. On this occasion no respiratory symptoms at all were seen.

Dopa absorption studies

After the i.v. administration of the labelled L-dopa 25% (controls 24 ± 4.7% M ± S.D., n = 10), and after the oral administration 6% (controls 6 ± 1.2%), of the radioactivity was recovered in the urine during the subsequent 3 days. From these figures it was calculated that 24% (controls 27 ± 5.9%) of the orally given L-dopa was absorbed. The plasma concentration of dopa after an oral dose of 0.5 g L-dopa never exceeded μmol/l, which corresponded to the values in control group (3.4 ± 3.1 μmol/l).

The plasma elimination curve of L-3,4-dihydroxyphenylalanine-1-^{14}C appeared exponential in the interval 60–180 min after the injection. The half-life was 56 min (controls 53 ± 5.0 min). The apparent distribution volume of the isotope, calculated from the theoretical concentration at zero time (obtained by extrapolation from the plasma disappearance curve in a semilogarithmic diagram) was 1.11 l/kg b.wt. (controls 1.59 ± 0.43 l/kg).

DISCUSSION

The patient's history, the clinical observations and the pharmacological studies by single-blind technique indicate that the L-dopa treatment was the cause of the respiratory disturbance. During the investigation anticholinergic treatment with benzohexolchloride was also given, and the possibility of a combined effect of L-dopa and the anticholinergic drug must therefore be considered. It is well known that toxic doses of anticholinergic drugs may produce tachypnoea. As far as is known, however, the type of respiratory disturbance reported here has not been observed as a side-effect of anticholinergic drugs. It had never occurred when the patient was treated only with the anticholinergic drug before L-dopa treatment. Furthermore, she revealed no other signs of anticholinergic side-effects. We therefore believe that the L-dopa treatment alone caused the respiratory disturbance observed.

The clinical picture with hypokinesia, rigidity, tremor and oculogyric crises did not differ from what is usually seen in parkinsonism caused by encephalitis. Thus the investigation did not reveal any other symptoms than the respiratory disturbance, which might indicate more advanced or widespread cerebral damage than that found in other patients with postencephalitic Parkinson's syndrome. Neither did our studies of the resorption and metabolism of L-dopa indicate that this patient differed from other parkinsonian patients in this respect. It is not improbable that the side-effect here described may exist in a certain number of L-dopa treated parkinsonian patients, although this patient is the first in our series of about 200 who has spontaneously reported the symptom. We therefore intend to look more carefully for this disturbance in other patients.

In connection with encephalitis lethargica many types of respiratory disturbance have been described, appearing as symptoms of the acute attack or as residua, but also as remote sequelae, sometimes being an important part of the general post-encephalitic picture (1, 6, 7). Turner and Critchley (7) state the existence of minor respiratory symptoms to be not uncommon if looked for in association with parkinsonism. Among the several disorders mentioned by these authors the dysrhythmia described as "apnoeic pauses" seems almost identical to the irregularity of respiration in our case. In view of this conformity it would seem reasonable to regard the encephalitis of our patient as a contributory cause of the respiratory disturbance, although she has not complained of such symptoms previously, not even during the acute phase of the disease. Then, on the other hand, it is all the more remarkable that this post-encephalitic symptom should be evoked by the L-dopa treatment at the same time as this reduces the other post-encephalitic symptoms, the parkinsonism.

ACKNOWLEDGEMENTS

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THE INCIDENCE OF ECG ABNORMALITIES IN ACUTE CEREBROVASCULAR ACCIDENTS

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Abstract. Based on an epidemiological study ECGs have been studied in 77 patients with cerebrovascular ac cidents. In the group with intracerebral hemorrhage ECG abnormalities of the T or U wave of the kind first described by Burch et al. were seen in 65% of all cases. In addition 16 of 17 patients in this group showed bnormal prolongations of the Q-T interval (aver age 1.4% of normal). ECG had been recorded in only 5 of 18 patients with subarachnoidal hemorrhage and consequently no firm conclusions can be drawn regarding the incidence of specific ECG abnormalities in this group. Four of the 5 patients, though, had prolonged Q-T duration. The incidence of T or U wave changes in the groups with thromboembolic disorders or transient ischemic ttacks as low (14%) or non-existent. Furthermore Q-T durations varied, the averages being only 106% and 105% of normal. Finally in the unspecified group, 28% showed T or U ave changes and rather marked Q-T prolongations. As discussed previously these cases may represent patients with undiagnosed intracerebral bleedings. In conclusion, ECG changes with marked increase of the Q-T interval and specific T or U wave changes ere most commonly found in patients with intracerebral hemorrhage, 94% of all showing abnormally long Q-T intervals while 65% showed T or U wave abnormalities as well.

In 1954, Burch et al. (2) described an ECG abnormality in patients with cerebrovascular accidents. Their finding in its most characteristic form may be summarized as a prolongation of the Q-T interval and a large and wide T or T U wave of the same general configuration as in myocardial ischemia. The most pronounced abnormality was seen in subjects with subarachnoidal or intracerebral hemorrhage. Later others have reported similar findings associated with spontaneous intracranial hemorrhage (3 6). Various suggestions have been offered in attempts to clarify the pathogenesis, e.g. electrolyte imbalance (2), subendocardial hemorrhage (3) and bleedings into the anterior fossa (6). A more likely explanation is that the cerebrovascular accident produces alterations of sympathetic tone with secondary ECG changes. This is supported by data from animal research. Thus, unilateral stellate ganglionectomy in 48 dogs produced ECG changes similar to those seen in patients with central nervous accidents (8).

The purpose of the present investigation was to study the ECG abnormality in a well defined population of patients with stroke, with particular emphasis on illuminating the incidence of this ECG abnormality in various forms of cerebro- ascular accidents.

MATERIAL AND METHODS

The present study was based on current epidemiological study of strok in Gothenburg (population 450 000), the details of hich have been outlined previously (5) In brief, all patients—65 years or younger—with diag nosis of cerebrovascular accident have been included in the epidemiological study as have all cases with death certificates indicating this disorder. Autopsy has been performed in all cases of death. A validity test of the epidemiological study showed that approximately 90% of all cases defined as above had been identified and included in the study (4)

The present study is based on subsample of the epidemiological study consisting of all cases included during the 6-month period between Nov 15 1970, and May 15 1971. During this period total of 111 patients (≤65 years) with acute cerebrovascular accidents were identified. There were 68 men (61%) and 43 women (39%), whose average age was 58 years (range 19–65) Six deaths occurred in non-hospitalized patients.

Of the 111 patients 18 were diagnosed as subarachnoidal hemorrhage (SAH), 77 as intracerebral hemorrhage (IH), 36 as thromboembolic disease (TE), 8 as transient ischemic attacks (TIA) and 22 as unspecified (NUD). Patients were assigned to the NUD group. g if spinal

Table I. *Electrocardiographic data*

Diagnosis	No. of pats.	No. of ECGs	Pats. with prolonged Q–T*	Average Q–T (% of normal)	Abnormal T(–U) wave	abnormal T(–U) waves
IH	27	17	16	124	11	65
SAH	18	5	4	113	2	40
TE	36	29	17	106	4	14
TIA	8	3		105	0	0
NUD	22	21	15	117	6	28

* > 105 % of normal.

DISCUSSION

In the present study the incidence of the characteristic ECG abnormality as described in the introduction was investigated in patients with cerebrovascular accidents who were all included in an epidemiological study of stroke. In agreement with others (2, 3), we found the highest rate of ECG abnormalities in patients with IH. This was true in regard both to T or U wave changes and to prolongation of the Q-T interval, which was significantly longer than in patients with TE or TIA. The incidence of T or U wave abnormalities in the IH group was 11 out of 17 or 65%. Sixteen patients in this group showed abnormally increased Q-T duration. Others have reported that this abnormality occurred in only 46 of about 10 000 consecutive hospital patients (7).

Unfortunately in the group of 18 patients with SAH, ECG had been recorded in only 5. Again prolonged Q-T duration was prevalent, but T or U wave abnormalities were seen in only 2 patients. Due to the small number of ECGs in this group we find it hard to draw firm conclusions regarding the incidence of T or U wave abnormalities in subarachnoidal hemorrhage.

As expected, there were no T or U wave changes in the TIA group and only a discrete increase of Q-T duration. This was true also for the TE group, but in this group the blind observer had classified 4 of 29 ECGs (14%) as having T or U wave abnormalities.

In the NUD group 6 ECGs showed T or U wave abnormalities, and these all had prolonged Q-T duration (mean 130%), thereby significantly increasing the average Q-T duration for the whole NUD group. As mentioned before, it is conceivable that patients with IH may have been classified as NUD in the absence of spinal puncture. Thus, it is possible that any or all of these 6 clearly abnormal cases were patients with intracerebral bleedings.

ACKNOWLEDGEMENT

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GENITOURINARY DISTURBANCES IN FAMILIAL AND SPORADIC CASES OF PRIMARY AMYLOIDOSIS WITH POLYNEUROPATHY

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Abstract. The occurrence of genitourinary disturbances has been analysed in 34 successive patients (24 males and 10 females) with familial or sporadic amyloidosis with polyneuropathy. Thirteen patients died, autopsy was performed on 9 of them. Out of 25 patients 9 had urinary retention and 10 both retention and incontinence. Advanced stages of urinary bladder dysfunction were characterized by loss of sensation of bladder fullness, reduced desire to void and difficulty in initiating the voiding. The incontinence usually had the character of ischuria paradoxa (overflow incontinence). Amyloid deposition occurred in the wall of the urinary bladder located in the nerves, vessel walls, and in the smooth musculature of the detrusor muscle. The function of the urinary bladder was obviously impaired by the affection of the nerves to the bladder and presumably also by the amyloid deposition in the detrusor musculature. Total impotence, occurring in 16 out of the 24 male patients, was considered to be due mainly to the amyloid affection of the nerves. Uraemia appeared in two patients only, signs of nephrotic syndrome in one of them. The amounts of amyloid deposits in the kidneys varied within wide limits. The substance could even be absent. Out of 9 cases examined glomerular deposits were found in 4, all of whom had proteinuria. Thirteen out of 28 patients examined had bacteriuria, 10 of them also proteinuria. The cause of proteinuria in this form of amyloidosis may be either amyloid deposition in the kidneys or infection of the urinary tract.

Familial occurrence of amyloidosis, indicating inherited predisposition to the disorder, has been observed during the last decades. As it has been described in an extensive account concerning amyloidosis (12), various clinical syndromes of heredofamilial amyloidosis have been reported. First described was familial amyloidosis with polyneuropathy appearing in Portugal (5, 19). This syndrome, clinically characterized by progressive peripheral neuropathy, has since been found elsewhere. Signs of involvement of the autonomous nervous system usually appear, such as genitourinary dysfunction, disturbance of the motility of the gastrointestinal tract, and postural hypotension. Constipation alternating with severe diarrhoea often occurs. Malabsorption has been found (2), leading to cachexia.

Patients with this form of amyloidosis often have urinary incontinence. So far this symptom has been interpreted as a sign of sphincter disturbance only (5, 7). In male patients impotence is an additional, common, and early manifestation of the neuropathy (2, 5, 7). These symptoms, as well as the histopathological changes in the urinary bladder, are only briefly mentioned in earlier reports. Available descriptions of kidney lesions are somewhat more detailed, although usually confined to statements about the localization and the degree of amyloid accumulation. Only exceptionally are more complete reports found concerning the histopathological lesions of the kidneys (19). In the North of Sweden about 50 cases of amyloidosis with polyneuropathy have been observed during the last few years. Most cases have been familial and some of them have been reported previously (2, 3, 4, 14). Some cases considered so far to be sporadic have had the same clinical symptoms.

One of the main purposes of the present report is to give a description of the clinical signs of urinary bladder dysfunction and impotence and to analyse histopathologically the urinary bladder lesions. Attention will be paid to the possibility that incomplete emptying of the bladder may predispose to urinary infection and secondarily to kidney lesions. Theoretically kidney

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Table I *Signs and symptoms of genitourinary lesions in 34 cases of amyloidosis with polyneuropathy*

Case	Sex	Age at examination (y.)	Duration of neuropathy (y.)	Neuropathy* (Legs)	Disturbance of micturition: Retention	Disturbance of micturition: Incontinence	Impotence	Urinanalysis: Proteinuria	Urinanalysis: Pyuria	Urinanalysis: Bacteriuria	Serum creatinine (mg/100 ml)
A 1	♂	66	16	+ + +	+	0	+	+	+	+	0 9
A 2	♀	63	10	+ + +	+	+		+	+	+	0.7
A 3	♂	50	10	+ + +	?	+					
A 4	♂	48	13	+ + +	+	0	+				
A 5	♂	70	24	+ +	0	?	+	0	+	+	0 9
A. 6	♂	64	6	+ + +	+	0	+	0	0	0	0 7
A 7	♂	68	18	+ + +	0	?	+	+	+	+	1 1
B 1	♀	60	4	+ + +	+	+		+	+	+	1.0
B 2	♀	68	5	+ + +	+	+		+	+	+	1.4
B 3	♂	46	6	+ + +	+	+	+	+	+	0	4.3
B 4	♂	60	3	+ +	+	+	+				
B 5	♀	64	7	+ + +	?	?					
B 6	♂	59	8	+ + +	+	+	+	+	+	+	0 9
B 7	♀	35	2	+	?	0					
B 8	♀	43	3	+	+	+	0	0	0	0	
B 9	♂	65	5	+ +	?	0		+	+	0	0.9
B 10	♀	55	2	+	?	0					
C. 1	♂	63	7	+ +	?	?	?	+	+	+	80 (NPN)
C. 2	♂	35	6	+ + +	+	0	+	0	0	0	0 8
D 1	♂	67	3	+ + +	+	?	?	+	+	+	0.8
D 2	♂	68	5	+ + +	+	?	?	+	+	0	1 0
D 3	♀	60	13	+ + +	?	0		0	+	+	
E. 1	♀	63	9	+ +	?	0		0	+	0	0.7
E. 2	♂	70	14	+ + +	?	0	?	0	+	+	1 2
F 1	♂	62	6		+	0	+	0	0	0	2 0
G 1	♂	58	3	+ +	?	0	+	0	0	0	0 8
	♂	51	2	+ + +	+	+	+	+	+	+	1 1
	♀	64	9	+ +	+	+		+		+	1.5
)	♂	38	2	+	+	0	+	0	0	0	0 9
	♂	57	4	+ +		0	0	+	0	0	1 1
	♂	57	7	+	+	+	+	0	0	0	1 1
M	♂	69	4	+	0	0	?	0	0	0	0 8
N	♂	61	16	+ +	0	0	+	0	0	0	0 9
0	♂	75	3	+	0	0	?	0	0	0	0 8

* + slight, + + moderate + + + pronounced.

lesions in these patients may be caused by infection or by amyloid deposits.

MATERIAL

The material consisted of our first 34 patients. T enty four of them are admitted to the Department of Medicine of the University Hospital, Umeå. The others were admitted to medical departments in other hospitals, but records were available for our study The patients have been listed in Table I. Familial cases are recorded with capital letters—indicating family—followed by figure (26 cases). The 8 cases recorded by capital letters only have so far been considered to be sporadic. The mean age at the time of examination was 58 (range 35–75) for males and 57 (range 35–68) for females. The verage interval from the appearance of the first toms of amyloid disease to the examinatic (range 2 24) for the men and 6 years (range 2–13) for the women.

The diagnosis of amyloidosis was established histopathologically in 30 cases by light-microscopical examination of biopsy or autopsy specimens. In 4 familial cases (A.3–4 B 4–5) no material was available for histopathological examination. They had, however clinical pattern characteristic of this type of amyloidosis Furthermore, the diagnosis of amyloidosis had been verified morphologically in some close relatives of these patients

Thirteen of the 34 patients (9 men, 4 omen) have died. Autopsy was performed on 9 (6 men, 3 women) The intervals from appearance of the initial clinical symptoms of amyloid neuropathy to death varied between 4 and 31 years (average 13). The mean interval between clinical e and death was 3 years (range 0–7) Those 4 were not examined post mortem (A.3–4 home or t a cottage hospital without mortem examination.

METHODS

Clinical grading

The peripheral neuropathy was graded semiquantitatively based on clinical manifestations as described in a previous report (3) (+ slight, 4 cases, ++ moderate, 10 cases; and +++ pronounced disturbances, 20 cases).

Urinalysis

The urine was analysed for proteinuria with Albustix® (Ames). Pyuria was considered to exist when urinary sediment, examined with an ordinary light microscope, contained more than 10 leucocytes/high-power field. A value of more than 100 000 bacteria/ml urine in consecutive specimens was considered significant for the presence of bacteriuria (16).

Autopsy and biopsy techniques

Six complete autopsies (A.2, A.5 B 1, E.1, G-1, H) were performed at the Department of Pathology Umeå, three (A.7 B.3 C.1) at other hospitals, and tissue specimens, fixed in formalin, were sent to our hospital. Table III indicates the tissue specimens taken at autopsy from urinary bladder and kidneys for histopathological investigation. In case A:5 altogether 7 transurethral biopsy specimens were taken from the urinary bladder over a period of 7 years owing to recurring papillary carcinoma of high differentiation.

The autopsy and biopsy specimens were fixed in 4% formaldehyde, dehydrated, and embedded in paraffin. The cut sections were routinely stained with van Gieson's stain. Alkaline Congo red (18) was usually used as amyloid stain. In case B 1 Thioflavin T (23) was also applied. Sections stained with alkaline Congo red were examined both in direct transmitted illumination and in polarized light (17). The sections stained with Thioflavin T were examined in a fluorescence microscope. Sections from urinary bladder ureters and kidneys were stained according to the Gram technique for demonstration of bacteria. PAS section as used in the search for fungi. Ladewig modification of the Mallory stain was also performed, mainly in order to detect any hyaline droplet degeneration. For similar reasons frozen sections of kidney specimens fixed in formalin were stained with Sudan IV.

RESULTS

Peripheral neuropathy

Table I includes the results of the clinical grading of the peripheral neuropathy appearing initially and most markedly in the legs.

Impotence

Total impotence occurred in 16 out of 4 male patients. In all of these 16 cases there were also other signs of affection of the autonomous nervous system. Two patients (B.9 and K) denied impotence, whereas information concerning this dysfunction was not certain in 6 patients (C 1 D-1 2, E.2, M O). As a first sign of incipient impotence the patients usually noticed loss of orgasm. Not until later did disturbance of erection appear One patient (B-6) observed loss of ejaculation as the first sexual dysfunction. Usually total and permanent impotence occurred before 50–55 years of age. Often impotence appeared early in the disease as in cases B 3 C.2, and J who had total impotence at the age of 35–40 years. Testicular atrophy penile involution or loss of pubic hair was not observed. There was no indication that the impotence was psychogenic.

Table II *Summary of urinanalysis in 28 cases of amyloidosis with polyneuropathy*

No. of pats.	Proteinuria	Pyuria	Bacteriuria
10	10	10	10
3	3	3	0
1	1	0	0
3	0	3	3
1	0	1	0
10	0	0	0
28	14	17	13

Disturbance of micturition

As shown in Table I, 10 patients had both urinary retention and incontinence. Retention occurred in a further 9 patients; 7 of them had no incontinence at the time of examination. Three patients were definitely free from disturbance of micturition, while information concerning retention or incontinence was insufficient in 14

The patients complained of various symptoms indicating impaired bladder emptying. Early in the disease sensation of bladder fullness and desire to void were retained. On micturition, however only small olumes were voided, giving a sensation of incomplete emptying. In a more advanced stage of the disease the patients had loss of sensation of bladder fullness associated with reduced desire to void. Interpreted as sign of reduced sensibility was the occurrence of retention of large volumes, sometimes 500–1 000 ml, without sensation of bladder fullness and without obvious desire to void Difficulty in initiating micturition often occurred The flow of urine was slow and poor Some patients had to use abdominal straining t initiate oding, while straining had an f ourable effect in others.

Table III. Post mortem findings of the kidney and urinary bladder in 9 cases of amyloidosis with polyneuropathy

Case	Age at death (y)	Duration of disease (y)	Histopathological findings: Kidney	Urinary bladder
A 2	64	15	Amyloid in vessel walls. Pyelonephritis	Amyloid in vessel walls, nerves and muscular layer. Inflammatory changes of the mucosa
A 5	77	31	No amyloid	Amyloid in vessel walls. Small accumulations of round cells, fibrosis
A 7	68	18	Amyloid in vessel walls, as lumps in medulla and capsule	Amyloid in vessel walls, nerves and muscular layer. Slight inflammatory changes of the mucosa
B. 1	61	5	Amyloid in vessel walls, glomeruli and tubular walls. Radial bands of round cells	Amyloid in vessel walls, nerves and muscular layer
B 3	48	8	Amyloid in vessel walls, glomeruli, and as lumps in the medulla. Accumulations of round cells adjacent to the renal pelvis	Amyloid in vessel walls, nerves and muscular layer. Slight inflammatory changes of the mucosa
C. 1	70	14	Amyloid in vessel walls and glomeruli. Small interstitial accumulations of round cells	
E 1	66	12	Spots of amyloid in the medulla	Amyloid in vessels walls and muscular layer. Slight inflammatory changes of the mucosa
G 1	60	5	Amyloid in vessel walls and spotted in the papillar tissue. Accumulations of round cells scattered interstitially and adjacent to the renal pelvis	Amyloid in vessel walls, nerves and muscular layer
H	53	4	Amyloid in vessel walls and glomeruli, as lumps in the medulla. Pyelonephritis	

Owing to retention 7 patients (A 1 2, 4 B 1 2, 4 H) had to be treated with an indwelling catheter The other 12 patients with urinary retention had residual volumes varying between 150 and 1 000 ml. When examined by catheter 5 patients were found to have no retention, while information concerning that symptom was uncertain in 10 cases.

In the 11 patients with incontinence overflow incontinence occurred in 6 men and 5 women, i.e. they had involuntary loss of urine when the bladder was distended. One female (B 8) with overflow incontinence had also stress incontinence. Patients A.2, B.1 2, 4 and H had incontinence at the time before an indwelling catheter was used. Dribbling after micturition was an uncommon symptom, appearing only in 3 males (B.3 B.6, L). Seventeen patients had no incontinence while information concerning this symptom was uncertain in 6 cases.

Serum creatinine

Only 5 (B.2, B.3 C.1 F 1 I) of the 26 cases examined in this respect had a creatinine content in serum of 1.4 mg/100 ml or more (Table I). The interval from the onset of neuropathic symptoms in these 5 patients ranged from 5 to 9 years. Four of them had signs indicating infection of the urinary tract. Patients B.3 and C.1 died of uraemia and heart failure. There was no clear relationship between duration of neurological symptoms and occurrence of increased values of serum creatinine. Thus the 21 patients with serum creatinine values of less than 1.3 mg/100 ml had symptoms of neuropathy for an average of 8 years (range 2–24). The creatinine values were

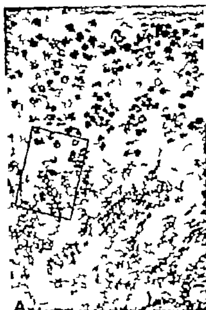


Fig. 1 (*A*) Case B 3. Low-power photomicrograph of renal tissue with abundant deposits of amyloid (black) in glomeruli. In addition there are deposits in the peripheral parts of the medulla (between dotted lines). These deposits are also abundant although less dense than the glomerular amyloid. Alkaline Congo red stain. 15



(*B*) Higher magnification of the area outlined in Fig. 1 *A* showing both amyloid deposits in the glomeruli (top) and in the medulla (bottom). The latter deposits surround small ... loops of Henle and collecting tubules. Alkaline Congo red stain. 45

normal in 6 of the 9 cases with duration of neuropathy for 10 years or more.

Urinalysis

The urine was examined for the occurrence of protein, leucocytes and bacteria in 28 of the 34 cases. Usually repeated examinations were performed. The findings are presented in Tables I and II. Signs of infection of the urinary tract appeared, with bacteriuria in 13 of these 28 cases (46%). On average, the patients with bacteriuria had longer duration of illness (11 years) than those without bacteriuria (6 years). Proteinuria was found in 14 cases (50%), most of them also had bacteriuria and pyuria. One patient had proteinuria only.

Five of the 13 patients with signs of urinary tract infection were treated by continual catheterization at the time of examination (A.1, 2, B.1, 2, H). In case B.6 proteinuria and infection occurred after cystoscopy. In patient A.5, who had pyuria and bacteriuria, repeated examinations with cystoscopy had been performed because of papillary carcinoma of the urinary bladder.

Enlargement of the prostate gland may cause retention and urinary tract infection also in these patients. The gland was found to be enlarged in some of the older males, but in only two was the enlargement noteworthy.

Gross haematuria occurred in 4 patients (A..., A.5, B.9, D.1). In case A.5 caused by bleedings from a papillary carcinoma of the urinary bladder. In the other cases no cause of the bleeding was detected.

Gross findings in genitourinary organs at autopsy

In most cases thorough descriptions were available of the relevant organs. No gross signs of amyloid deposits were recorded, neither for kidneys nor for other genitourinary organs examined.

In three cases (A.2, A.5, G.1) the weights of the kidneys were within normal limits, whereas in case E.1 the kidneys were somewhat diminished in size and in case B.1 their combined weight was only 170 g. The kidneys of case H weighed 590 g together. They were flabby and



Fig. 2. Case B.3. Photomicrograph demonstrating small deposits of amyloid (black) in nerves in the urinary bladder. The nerves, running horizontally and being slightly tortuous, are located in the muscular layer of the urinary bladder as is illustrated by the occurrence of smooth musculature in the upper and lower parts of the Figure. Alkaline Congo red stain. 115

contained several small abscess-like foci, especially in the papillae.

Inflammatory changes with oedema, hyperaemia and haemorrhagic lesions were found in the mucous membrane of the renal pelvis and ureters (case H) and urinary bladder (A.2, A.5, ... H). Otherwise no obvious signs of infection were seen macroscopically in these organs.

In case A.5 no recurrence of the papillary carcinoma could be detected in the urinary bladder.

Histopathological observations in genitourinary organs

Amyloid deposits in the kidneys. As regards the kidneys, autopsy specimens from 9 patients were examined (Table III). The amounts of amyloid deposits in the kidneys varied within wide limits and in one case (A.5) there was no amyloid in the renal tissue.

Glomerular amyloid occurred only in 4 cases. In 2 of these the deposits were slight to moderate (B.1, H) and in the other 2 (B.3, C.1) the glomeruli were severely affected (Fig. 1 *A*).

In 7 cases amyloid deposits appeared in vessel walls in the cortex, mainly small vessels were affected, although small deposits could occasionally be seen also in the walls of larger vessels. Usually the number of affected vessels, varying from a few to numerous, agreed fairly well with the degree of glomerular lesions.

Patients with glomerular deposits seemed to have the most advanced deposits in the medulla. In 3 cases (B.1, B.3, C.1) with glomerular amyloid, deposits of amyloid appeared in the boundary between the cortex and the medulla (Fig. 1 *B*). These accumulations of amyloid were the result of fusions of amyloid rings surrounding small vessels, loops of Henle, and collecting tubules. Deposits occurring in the middle and distal parts of the medulla were usually more solid, and often pierced by only one cannular structure, a blood vessel or a collecting tubule.

Other degenerative lesions in the kidneys. With Ladewig's modification of the Mallory stain and with the PAS reagent no marked hyaline droplet degeneration was seen. Neither were any changes of fibrinoid necrosis demonstrated. In examination of frozen sections only a few small foci of sudanophil droplets were observed interstitially and in the lumina of some tubules in some of the cases. Only in cases B.1 and B.3 were sudanophil droplets found in the epithelium of some tubules.

Amyloid deposits in other genitourinary organs. In all 7 cases examined (Table III) moderate to abundant accumulation of amyloid was found in vessels of the wall of the *urinary bladder*. Except in A.5 and B.1 amyloid was also found in the nerves of the bladder (Fig. 2). In the connective tissue of the submucosa no amyloid or only small amounts of it were usually seen. Only in case G.1 were there more abundant deposits.

In the detrusor muscle there were slight (A.2, A.5) to more abundant (A.7, E.1, G.1) deposits of amyloid in some muscle bundles. Moderate amounts of amyloid were found in most muscle bundles in cases B.1 and B.3. The deposits often had the form of lumps in peripheral parts of the muscle bundles (Fig. 3 *A*). Transversely sectioned bundles showed that small amounts of amyloid appeared as fine rings around each smooth muscle cell (Fig. 3 *B*). When the amyloid rings were thicker, some atrophy of the muscle cells was found. In more extensively affected areas the musculature seemed to be almost totally replaced by amyloid.

In the biopsy specimens from the urinary bladder of the patient with a papillary carcinoma (A.5) no amyloid was found in the stroma of the tumour. The substance was, however, demonstrated in the walls of blood vessels in the submucous connective tissue of the bladder.

The *ureters* were examined only in case B.1

Amyloid was found adjacent to smooth musculature.

In cases A.2 and B 1 amyloid appeared in vessel walls in the *ovaries*. Also in the *Fallopian tubes* and in the *uterus* the main part of the deposits were found in vessel walls, although amyloid was observed also in nerves and adjacent to the smooth musculature. In the uterine cervix of case B 1 there was amyloid in collagenous connective tissue and in vessel walls. The substance had a similar distribution in the *vagina*, where it also occurred in a few nerves.

In the parenchyma of the *testis* (case G 1), amyloid deposits occurred only in walls of small blood vessels. In addition, large amounts of amyloid were found in the connective tissue of the fibrous capsule of the testis. No clearly pathological changes were observed in the number or structure of the Leydig cells. A patchy atrophy of the germinative cells was seen, with hyalinization of the testicular tubules. As a rule however complete spermatogenesis was present or only a slight atrophy was seen.

The amyloid deposits in the *epididymis* appeared mainly in the walls of small vessels, whereas the sparse deposits in *ductus deferens* were mainly located adjacent to smooth muscles. Small to moderate amounts of amyloid were demonstrated in the small nerves in the tissue surrounding the epididymis and ductus deferens. Abundant stromal deposits were seen in the *prostate* in both examined cases (G 1 A.7). In case A.7 several small nerves of the gland showed amyloid deposits.

Signs of infection. In two cases (A.2, H) a characteristic cellular picture of acute pyelonephritis was seen, with occurrence of radial rays of granulocytes. In 3 cases (B 1 C.1 G 1) an increased number of lymphocytes and plasma cells occurred in the cortex, either in radial rays or around glomeruli. These lesions could either represent a mild chronic pyelonephritis, be related to ischaemia or even to the amyloid deposits. Another case (B 3) showed a mild chronic pyelitis, but there were only few lymphocytes in the kidney. In the remaining 4 cases no signs of pyelonephritis were seen.

The mucous membrane of the urinary bladder showed signs of inflammation in case A.2. Mild inflammatory changes were also found in cases A 7 B.2 and E.1



Fig. 3 (A) Case B.3 Low-power photomicrograph showing the occurrence of deposits of amyloid in the smooth musculature of the detrusor muscles. There is some tendency for the deposits to be located in peripheral parts of the muscle bundles. *Alkaline Congo red stain.* ×45.

(B) Higher magnification of transversely sectioned muscle bundles in the urinary bladder of the same case. Individual muscle fibres and delicate membranes surrounding each fibre can be identified. Around some muscle fibres these membranes are thickened due to amyloid deposits. Alkaline Congo red stain. ×290

In no case were any signs of fungal infection observed, neither in the kidneys, nor in the urinary bladder. Using the Gram technique, bacteria were only exceptionally demonstrated in the kidneys and hardly at all in the urinary bladder. Occasional foci of bacteria were seen in tubules (A.5 B 1), collecting tubules (A.5 B.3) blood vessels (G 1) and in a glomerulus (A 7). There was no distinct inflammatory cellular reaction around these few foci of microorganism.

COMMENTS

The material

This report was intended to present an analysis of some genitourinary lesions in the hereditary type of amyloidosis with polyneuropathy but the sporadic cases were included as it did not seem possible to draw a sharp line between the two forms. Thus there were no apparent clinical or histopathological differences between patients designated as familial and sporadic in the present report. It must also be stressed that not even a common family history makes a reliable differential diagnosis possible. Thus, of the cases originally considered sporadic in an earlier report (2) some have subsequently been shown to be hereditary after a thorough genealogical analysis. The following discussion does not include reports that are confined to descriptions of cases with sporadic amyloidosis.

Tumours and amyloidosis

Concerning the present material the case with a papillary carcinoma of the bladder also requires comment, as some malignant tumours are known be associated with amyloid (12). It might per- also seem remarkable that in precisely that amyloid was found in the bladder wall but not in the renal tissue. There was, however, no amyloid in the stroma of the tumour. Furthermore, the patient had characteristic neurological symptoms, similar to those of some relatives with overt amyloidosis.

The genitourinary neuropathy

It is apparent both from the present analysis and other reports concerning hereditary amyloidosis with polyneuropathy (5–7) that impotence is an early and common manifestation of the disease. The disturbance of micturition, as described, also appears early in the disease, presumably just as early as symptoms of peripheral neuropathy. This disturbance may easily be overlooked, especially in the early stage of the disease. The patients are often not aware of the affection of the urinary tract or not particularly troubled by it.

The impotence and the urinary bladder retention in combination with the patient's loss of sensation of bladder fullness, are all manifestations which in many respects are similar to the genitourinary disturbances occurring in diabetes mellitus (8). In this form of amyloidosis, however, the disturbances seem to progress more frequently to more advanced stages than in diabetes. This may be due to the fact that in amyloidosis the deposition of amyloid may occur not only in blood vessels and nerves but also adjacent to smooth muscle cells, implying an additional impairment of various functions. Thus the bladder function may be impaired by amyloid deposits in the detrusor musculature. Histopathological findings in the urinary bladder have been described only exceptionally (19) and studies of bladder function have not been met with in previous reports about familial amyloidosis with polyneuropathy. Whether the disturbance of bladder function is due only to affection of the nerves, or whether deposits in the detrusor muscles in some way may influence the function, remains an open question.

In some cases of amyloidosis with polyneuropathy in Portugal, more advanced testicular lesions were found post mortem (19) than in our case G 1. In biopsy specimens from patients with impotence, however, the amyloid deposits were confined to vessel walls. Spermatogenesis was not abolished, and the Leydig cells had a normal morphological appearance (19). Abundant deposits of amyloid were found at autopsy in the pelvic nerves and ganglia (20), favouring the supposition that the impotence is mainly of neurological origin.

The occurrence of amyloid deposits in the ovaries has been described earlier in this form of amyloidosis (15) but the distribution of amyloid in the Fallopian tubes and vagina has not been studied previously. The observations made in the present cases illustrate the wide distribution of amyloid deposits that may occur in the genital organs as well.

Urinary tract infection

It is a well known and generally accepted fact that the occurrence of urine retention and the introduction of instruments in the urinary bladder via the urethra both imply increased risks of a urinary tract infection. It seems evident, therefore, that the disturbed urinary bladder function in many of our patients, as well as catheterization in some of them, has contributed to the

high frequency of bacteriuria (13 out of 28 patients).

Only sparse information is available in the literature about the occurrence of urinary tract infection in familial amyloidosis with polyneuropathy. In two Japanese cases pyelonephritis was considered to be a contributory cause of death (6). Although no details were given, it was also stated that urinary tract infection occurred in members of an American family with neuropathic amyloidosis (22).

There is no close correlation between the occurrence of bacteriuria and the histopathological findings of bacterial inflammation in the kidneys and urinary bladder in our material. This may be due to the fact that the two types of examinations were performed at different times. Therapy with antibiotics just before death may also have altered the picture.

Proteinuria

It has been stated that varying degrees of proteinuria always accompany glomerular amyloidosis, apparently due to basement membrane lesions evoked by the glomerular amyloid deposits (9). In the present material all 4 cases with amyloid found in the glomeruli had proteinuria. Sometimes, however, amyloid may occur in the kidneys—even in the glomeruli—without obvious proteinuria (21, 24). In two of our cases without proteinuria amyloid was found in the renal medulla (E.1, G.1) and in vessel walls (G.1) but not in the glomeruli.

According to the results of this study the occurrence of proteinuria in this form of amyloidosis may be due not only to amyloid deposition in the kidneys but also to infection of the urinary tract.

Nephrotic syndrome

In various types of amyloidosis with renal involvement a nephrotic syndrome appears in connection with extensive glomerular amyloidosis (1). This statement is illustrated by our case with a nephrotic syndrome (B.3), whose glomeruli were heavily infiltrated with amyloid.

It is apparent that lipid histochemical findings in the kidney should be interpreted with caution. Thus there are many controversial opinions about what is pathological and what is normal in this respect, except for the fact that the occurrence of sudanophilic droplets in the interstitial tissue of the medulla is considered a normal finding in post mortem examinations of older patients (25). In our patient with a nephrotic syndrome (B.3) lipids were found in some tubular cells.

Haematuria

Patients with renal amyloidosis may have persistent haematuria (12). One of our patients had bleeding papilloma of the bladder. He had amyloid in the mucous membrane of the bladder but no amyloid in the kidneys. In 3 other patients no obvious cause of their haematuria could be detected. In one of them autopsy revealed amyloid in vessel walls in the kidneys but no amyloid in the glomeruli.

Signs of general haemorrhagic diathesis were not noticed in the patients with haematuria. Bleeding of the skin did not appear in any of these 4 cases either.

Amyloid deposits in the kidneys and renal failure

It is well known that amyloid deposits may occur in the kidneys in neuropathic amyloidosis (19). Generally the same degree of variation from case to case, as in the present study, has been found concerning the amount of amyloid deposits. Our findings that glomerular deposits were coexistent with depositions in the boundary between the cortex and the medulla in cases with established heredity may be typical. Similar findings were described for three Portuguese cases (19) and one German case (13).

The frequency of clinical signs of renal failure may differ from material to material. Thus in a large Portuguese series a nephrotic syndrome or a severe renal insufficiency occurred only exceptionally (10, 11), whereas in a few American cases uraemia was the most common cause of death (22). In the American patients severe amyloidosis of the renal arteries was assumed to be the cause of extensive renal atrophy and contraction rather than deposits in the arterioles or glomeruli. Moreover, they seemed to have more extensive amyloid deposition in other solid viscera, e.g. the liver and the spleen, than we have found in our cases. Thus, concerning the affection of the kidneys, the findings in our material seem to be more similar to those of the Portuguese cases.

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SARCOIDOSIS AND MYELOMA OF LAMBDA TYPE IgG

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Abstract. This report is concerned with the combination of chronic sarcoidosis and myeloma. The myeloma, of lambda-type IgG, was diagnosed 6 years after the onset of sarcoidosis. A gradually increasing ESR during the interval provided an indication of gradual development of the plasma cell dyscrasia. Signs were observed of depressed cellular immunity most probably attributable to the sarcoid disease. Phytohaemagglutinin-induced lymphocyte transformation in vitro was markedly depressed. In comparison with earlier test results the tuberculin skin reaction was depressed. The in vitro response, with PPD as stimulant, was compatible with the skin reaction. Lymphopenia was present, but the ratio of T and B lymphocytes was normal. A discussion bears upon the discrepancy between the poor PHA response and the normal number of T lymphocytes, and the possible relationship between sarcoidosis and myeloma, as compared with known disorders associated with monoclonal paraproteinaemia.

Sarcoidosis is characteristically associated with depressed delayed-type hypersensitivity reactions, whereas the humoral antibody response is normal or even more marked than normal (16). As a rule normal or slightly increased concentrations of serum immunoglobulins (Ig) have been reported (11, 13). A few sarcoidosis patients have been recognized in studies on the occurrence of serum M components in various diseases (6, 15).

The combination of sarcoidosis and myeloma has not been reported previously. Of course, this may be a coincidence. However prolonged antigenic stimulation, for example by autoimmunization, tumours and chronic infections, may give rise to monoclonal plasma cell proliferation (10, 22). A simultaneous immune deficiency state may increase the susceptibility to development of monoclonal gammopathy (1). As sarcoidosis is associated with depressed cellular immunity it is of specific interest to report on a patient with long-standing chronic sarcoidosis and a myeloma developing simultaneously.

CASE REPORT

Female, born in 1918, the youngest of 13 children. Her mother died at the age of 54 from chest disease, probably tuberculosis. One brother had died of pulmonary carcinoma, and another of gastric carcinoma.

Within the period 1949–60 the patient was hospitalized on four occasions for mental disturbances. In 1966 she was subjectively in good health and symptom-free when she took part in a compulsory mass-radiographic survey. Three years earlier the chest X-ray had been normal. She underwent examinations in hospital three months subsequently. The chest X-ray was unchanged; no parenchymal infiltrations were visible. Peripheral adenopathy was not noted, the liver and spleen were not palpable. Her tuberculin skin test was negative to 0.1 TU of PPD (tuberculin units of purified protein derivate) but positive to 1 TU. TB cultures of sputum were negative. ESR was 6 mm/h, the peripheral blood picture normal. Serum calcium was 9.6 mg/100 ml. A prescalenic lymph node, obtained by means of Daniels' method, showed epithelioid cell granulomas. Treatment with corticosteroids was not considered necessary.

During the years 1967–69 the patient had follow-up examinations at a tuberculosis dispensary. No changes were at any time apparent in the chest X-ray but an increasing ESR was noted (8–12–15–25–37 mm/h).

In 1970 the patient observed deterioration of vision. Ophthalmological examination revealed bilateral uveitis, and she was treated with topical applications of cortisone. In 1971, despite continued therapy severe posterior synechiae were observed.

In April 1972 the patient again participated in a mass-radiographic survey in another part of the country. The bilateral hilar lymphoma syndrome was noted, and she was admitted to the Ostersund Hospital for further examination, and subsequently to the Fourth Department of Medicine, Helsinki University Central Hospital.

The clinical examination revealed a slightly overweight patient (height 163 cm, weight 70 kg). A papular lesion, 0.8 × 1.5 cm, was observable on her left cheek; else-



Fig. 1. Chest X-ray illustrating the markedly enlarged hilar lymph nodes.

where the skin was normal. Peripheral lymph nodes, spleen and liver were not palpable. Heart and lung auscultations were normal. BP was 170–190/110–120 mmHg.

The chest X-ray showed markedly enlarged hilar lymph nodes (Fig. 1). The enlargement was of the same degree as that in 1966. No parenchymal infiltration were noted. The ventilatory function was normal. The ECG was normal.

Tuberculin skin tests were now negative to 1 TU of PPD but positive to 10 TU. The Kveim test was positive (histological confirmation). Changes characteristic of sarcoidosis were found on histological examination of the skin lesion. A biopsy specimen of the hilar lymph nodes, obtained by mediastinoscopy, displayed fibrotic granulomatous lesions; no plasma cells were found in this specimen.

During a period of eight months in 1972 the ESR gradually increased from 42 to 95 mm/h. In Nov. 1972 the Hb concentration was 12.9 g/100 ml, and the platelets 272 000/μl. The leukocyte count varied between 3 600 and 5 200/μl, of which 8–34% were lymphocytes. The total number of lymphocytes was always below 1 300/μl. Up to % of plasma cells were found in the peripheral blood. The liver enzyme tests were normal (S-GOT 5 U/l, alkaline phosphatase 49 U/l). Normal values were found for serum calcium (10.0 mg/100 ml), urinary calcium (248 mg/24 h), serum phosphorus (3.5 mg/100 ml) and serum creatinine (1.0 mg/100 ml). The urinary sediment was normal; the reactions for protein and glucose were negative. Bence-Jones protein was not detected in the urine. I view of the slight arterial hypertension, i.v. pyelography was performed. This displayed normal conditions, except for a double pelvis on the right side.

The bone marrow specimen displayed normal erythropoiesis, granulocytopoiesis and megakaryocytopoiesis. An increased number of plasma cells was found, amounting to 15% of all nucleated marrow cells. The lymphocytes were normal. No lesions suspected of multiple myeloma were found on radiological bone survey, but cystic lesions compatible with sarcoidosis were visible in three phalangeal bones of the hands.

The serum total protein concentration was increased (ad 9.2 g 100 ml). The electrophoretic determinations showed paraprotein in the γ-globulin fraction (Fig. 2). The concentrations of the fractions in the first determination (total protein 7.8 g/100 ml) were as follows: albumin 3.43 g/100 ml, α_1-globulin 0.39, α_2-globulin 0.62, β-globulin 0.47, γ-globulin 0.86 and paraproteinaemic fraction 2.03 g/100 ml. The immunoelectrophoretic investigations disclosed rapid paraprotein of lambda-type IgG

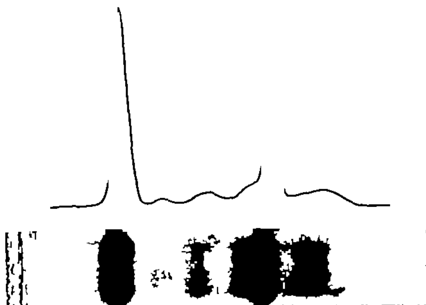


Fig. 2. Electrophoretic pattern of the serum proteins.

(Fig. 3). Quantitative determination of the serum immunoglobulins by radial immunodiffusion technique ga the following results: IgG 5 920, IgA 340 and IgM 80 mg/100 ml. The concentrations of serum complement are normal; C 3 126, C 4 38 mg/100 ml. Tests for rheumatoid factor in serum were negative. The antistreptolysin and antistaphylolysin titres were normal. The direct Coomb's test as negative, and cryoprecipitation was not demonstrated. Immunofluorescent nuclear antibodies were demonstrated in low titres with specific IgM antiserum (1 80), but not with IgG antiserum. No antibodies to thyroglobulin, gastric parietal cells, smooth muscle, glomerules and mitochondria were detected.

As mentioned above, increased numbers of both mature and immature plasma cells were found in the differential counts. The number of WBC in DNA synthesis as determined by means of tritiated thymidine labelling immediately after withdrawal of the blood sample (21). The number of labelled cells in the autoradiograms was 2.2% of the lymphoid cells, more than 10 times the number found in healthy controls (21). Many of the labelled cells differed morphologically from the atypical lymphocytes found, for example, in viral infections, and are probably plasmablasts.

For further study of the patient's lymphocytes, in vitro cultures er prepared on four occasions by method described previously (21). Phytohaemagglutinin (PHA)-induced lymphocyte transformation as markedly depressed; in 3-day cultures with 20% autologous plasma, only 7 to 10% of the lymphoid cells were blasts, and in cultures with control human plasma or foetal calf serum the esponse was even smaller. With PPD as stimulant the response was on the average 8% blasts in 6-day cultures; this is compatible with that normally observed in subjects with positive skin eactions to 10 TU of PPD (19). In control cultures without added stimulants no background proliferation occurred and the number of plasma cells as not higher than that found in cultures from healthy subjects (1–2 plasma cells/1 000 lymphocytes in 6-day cultures).

The ratio of thymus-dependent lymphocytes (T cells) to thymus-independent lymphocytes (B cells) was estimated

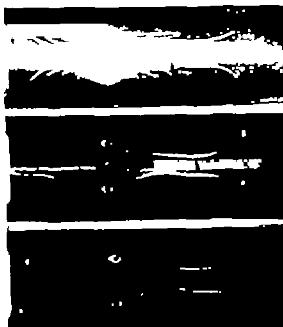


Fig. 3. Immunoelectrophoretic bands obtained with (beginning from the top) patient's serum and normal serum precipitated ith antihuman serum, patient's serum and IgG antiserum, patient's serum and antilambda light chain serum.

by determination of the formation of non-immune rosettes with sheep erythrocytes, and of the rosettes formed by lymphocytes carrying C'3 receptor (8). With these techniques the patient's peripheral lymphocyte population consisted of 84% of the former and 14% of the latter type.

The patient is subjectively in good health. Th gradually increasing ESR, the hyperproteinaemia with paraproteinaemic fraction exceeding 2 g/100 ml, and the presence of immature plasma cells in the peripheral blood, indicate presymptomatic myeloma. Consequently treatment with melphalane has been instituted.

DISCUSSION

The patient fulfilled the diagnostic criteria of sarcoidosis; the multisystem involvement of typical organs (lymph nodes, eyes, skin and phalangeal bones) histological support of sarcoidosis in specimens from lymph nodes and the skin and a positive Kveim test.

The serum proteins have been the subject of several studies concerned with sarcoidosis. Electrophoretic investigations have demonstrated increased α_2-globulin in acute sarcoidosis with erythema nodosum (7 12, 17) whereas elevated levels of γ-globulin have been considered charac[illegible]istic of the chronic stage of the disease (9). [illegible]ever with many patients the electrophoretic [illegible] yields variable results, which bear a poor correlation with the stage and activity of the disease. In acute sarcoidosis, particularly in combination with erythema nodosum, a tendency has been noted to increased concentrations of IgM (11 13). In other sarcoid patients, although generally increased values have been noted, no specific alterations of serum immunoglobulins have been found.

In an extensive study relating to the occurrence of serum M components, Hallén found six sarcoidosis patients (6). A 63-year-old female had pulmonary sarcoidosis and an M component of IgG simultaneously A 73-year-old female had had sarcoidosis 6 years before detection of an M component of IgG and a 64-year-old female had had sarcoidosis 5 years before an M component of IgA was found. At autopsy three other patients with serum M components were found to have sarcoidosis; in two of them it was widespread. The concentrations of all these paraproteinaemic fractions were low and they were classified as benign monoclonal gammopathies. Riva found two cases of sarcoidosis among 33 patients with "idiopathic paraproteinemia" (15). No further details were provided. One patient with sarcoidosis and macrocryoglobulinaemia has also been reported (20).

The depressed delayed-type hypersensitivity in sarcoidosis is clinically demonstrable as a depressed tuberculin skin sensitivity which correlates with the in vitro reactivity when the blood lymphocytes are cultured in the presence of PPD (5) In some cases a diminished in vitro response to PHA has also been observed (5). The case reported had obvious signs of depressed cellular immunity The poor PHA response is most probably attributable to the sarcoidosis and not to the monoclonal plasma cell proliferation, as a normal transformation rate has been noted in both untreated and treated patients with multiple myeloma (3). The high number of lymphocytes bearing receptors for sheep erythrocytes is an interesting finding in combination with the markedly depressed PHA response. It is believed that both the sheep erythrocyte rosette formation and the PHA responsiveness represent characteristics of thymus-dependent lymphocytes (8). This discrepancy may have its explanation in the PHA-induced lymphocyte transformation and the rosette formation with sheep erythrocytes, measuring different characteristics of lymphocytes, possibly representing different subpopulations of T cells.

Although the paraproteinaemic M component was recognized for the first time 6 years after the onset of sarcoidosis, the gradual increase in ESR during the period of 6 years most probably indicates that the M component did not suddenly develop in 1972. No skeletal lesions compatible with myeloma were radiographically visible, but the increasing ESR, terminating in a paraproteinaemic fraction in excess of 2 g/100 ml, and the occurrence of immature plasma cells in the peripheral blood, indicate a developing myeloma and do not favour the assumption of so-called benign monoclonal gammopathy

A recent report has suggested that sarcoidosis is accompanied by a diminished number of T cells and an increased number of B cells, and that the changed ratio might be of pathogenetic significance (2). The case reported had a normal ratio of T and B cells, but nevertheless displayed signs of depressed delayed-type hypersensitivity and marked stimulation of the B cells terminating

in monoclonal plasma cell proliferation. Accordingly the findings do not fit the hypothesis.

However monoclonal gammopathy may arise secondary to prolonged antigenic stimulation induced by autoimmunization, as in rheumatoid arthritis, Sjögren's syndrome and related disorders (22), or by tumours and chronic infections (10). In combination with immunological deficiency monoclonal gammopathy has been observed in patients with pernicious anaemia (1 18). In experimental models apparent viral infections, known to depress the cellular immunity induce autoimmune diseases frequently terminating in monoclonal gammopathies (4 14). The chronic sarcoidosis of the patient was accompanied by depressed cellular immunity It thus seems attractive to suppose that this immunodeficiency might have contributed to development of the monoclonal paraproteinaemia.

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SUPPRESSION OF FACTOR VIII ANTIBODY BY COMBINED FACTOR VIII AND CYCLOPHOSPHAMIDE

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Abstract. Four patients with severe haemophilia A complicated by inhibitors of factor VIII (corresponding to 0.5, 0.7, 1.0 and 2.5 U/ml plasma) and a non-haemophilic woman with an acquired inhibitor of factor VIII (corresponding to 160 U/ml plasma) have been treated with a large single dose of factor VIII concentrate (4 000–8 000 U factor VIII) and cyclophosphamide in connection with severe bleeding episodes. In three of the haemophiliacs this treatment had a good haemostatic effect, it immediately raised factor VIII level to about 50% and suppressed the antibody titre. The inhibitor level remained at zero for 5–10 days, after which it began gradually to return toward its original level. No secondary rise in antibody titre was seen. In the fourth haemophiliac the factor VIII level could only be raised to 8% and a marked secondary increase occurred in antibody titre. In the woman with an acquired inhibitor of factor VIII in extremely high titre it was not possible to neutralize the inhibitor, but constant suppression of antibody titre was achieved. Patients with haemophilia A and inhibitors and non-haemophiliacs with acquired inhibitors of factor VIII can thus be treated with advantage with a large dose of factor VIII concentrate combined with cyclophosphamide, provided factor VIII is administered in doses large enough to neutralize the inhibitor and to raise factor VIII level to about 50%. Although it is not possible to prevent the production of antibody, such treatment can suppress the production long enough to permit effective substitution therapy in connection with severe bleeding episodes and surgical procedures.

The presence of inhibitors of factor VIII is a dreaded complication. Inhibitors have been shown to occur with a frequency of 6–21% in severe haemophilia A (2, 10, 15, 22). These inhibitors are as a rule γ-G globulin antibodies specifically directed against factor VIII (20). Antibodies directed against factor VIII may also occur spontaneously in patients with collagen diseases, drug reactions, certain neoplasms and other disorders (1, 6, 13). Most patients with acquired anticoagulants have a haemorrhagic diathesis as severe as that observed in the most severely affected haemophiliacs.

Patients with inhibitors are known to be refractory to treatment. Infusion of factor VIII concentrates in patients with inhibitors is usually followed by a marked rise in the titre of inhibitors within a few days, after which further doses are useless. Exchange transfusions combined with substitution therapy have been tried in cases with life-threatening bleeding (8, 19). Other measures to control the antibodies have consisted mainly of long-term treatment with corticosteroids or immunosuppressive drugs (1, 3, 7, 11). Dormandy et al. (5) produced inhibitors of factor VIII in monkeys by injecting porcine factor VIII. Development of these inhibitors could be prevented by simultaneous immunosuppressive therapy with cyclophosphamide, azathioprine or chloramphenicol, provided that it was started before the inhibitor had developed.

Green (7) recently described a female who had psoriasis and an inhibitor of factor VIII, and in whom the inhibitor was suppressed by simultaneous administration of an i.v. dose of cyclophosphamide and a large dose of factor VIII. Lusher et al. (12) described two patients who had haemophilia A and antibodies against factor VIII and in whom a single dose of factor VIII concentrate and cyclophosphamide suppressed factor VIII antibody production. Lechner et al. (11) treated two patients with haemophilia A and antibodies with factor VIII concentrate and azathioprine, but they were not able to prevent the increase of inhibitor titre. Nilsson et al. (16), however, reported two patients with haemophilia B and inhibitors of factor IX, in whom the inhibitor

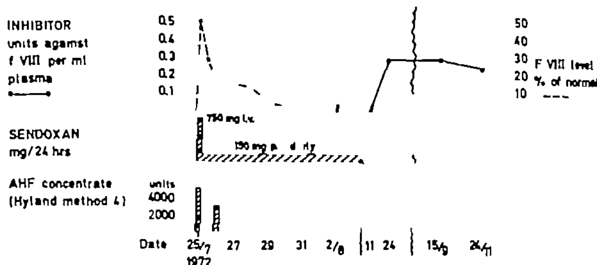


Fig. 1 Case 1 (15 years, weight 53 kg, severe haemophilia A) Course and treatment.

was suppressed and haemostasis achieved by simultaneous administration of cyclophosphamide and a large dose of factor IX concentrate

This paper reports four patients with haemophilia A and inhibitors of factor VIII and one patient with collagen disease and an acquired ... of factor VIII, who were treated by ... ultaneous administration of factor VIII con... and cyclophosphamide. In three of the cases the production of the inhibitor was temporarily suppressed and haemostasis was achieved. In the two cases that did not respond it was not possible to administer factor VIII in amounts sufficient to neutralize the inhibitor

METHODS AND MATERIALS

Coagulation tests. The *factor VIII activity* of plasma and concentrates was assessed from its normalizing effect on the recalcification time of platelet-rich haemophilia A plasma containing less than 1% factor VIII of normal. The amount of factor VIII present was expressed as a percentage of that found for normal standard consisting of pooled plasma from ten healthy individuals (14).

Inhibiting effect of patient' plasma on the recalcification time of normal plasma (simple anticoagulant test). Normal plasma, 0.2 ml, and 0.2 ml of the plasma to be tested in various dilutions were incubated for 3 min at 37°C. 0.2 ml 0.03 M $CaCl_2$ solution was then added, and the clotting time was determined. The highest dilution of the patient's plasma that prolonged the recalcification time was taken as the anticoagulant titre

Quantitative determination of inhibitor of factor VIII Various dilutions (1/1, 1/2, 1/5 and 1/10) of the plasma to be tested (0.8 ml) are incubated with 0.2 ml of concentrate of factor VIII (3 U factor VIII/ml) at 37°C for 2 hours. As a blank 0.6 ml saline was incubated with 0.4 ml of the factor VIII concentrate Following incubation the blank and the mixtures of plasma and factor VIII concentrate were assayed for residual factor VIII activity in the way described above The inhibitor activity of the plasma was expressed as the number of units of factor VIII (1 U of factor VIII is defined as the amount of factor VIII present in 1 ml normal plasma) inactivated by 1 ml of the plasma

Determination of AHF-related antigen. AHF-related protein in plasma was determined immunologically by electrophoresis in agarose gel containing antibodies in the way described by Holmberg and Nilsson (9). The antiserum against factor VIII was prepared as reported by Holmberg and Nilsson (9). A pool of normal plasma from 20 healthy individuals was used as standard.

Factor VIII concentrate. 1) Human fraction I-0 prepared by Kabi (AHF Kabi) according to the glycine method of Blombäck and Blombäck (4). The factor VIII activity of one bottle (100 ml) corresponds approximately to that of 300 ml fresh human plasma. 2) Antihaemophilic factor (Hyland method 4). The factor VIII activity of one bottle (7 ml) corresponds to that of 150–220 ml fresh human plasma.

CASE REPORTS AND RESULTS

Case 1

A boy born in 1957 with severe haemophilia A. When first seen in Malmö in 1958 the factor VIII level was 0.1%. In 1961–68 the patient received altogether 33 bottles of AHF Kabi and 1 bottle of blood in association with joint bleedings, large haematomas and tooth extractions. In 1968 it was found that factor VIII rose to only 5–10% after administration of doses calculated

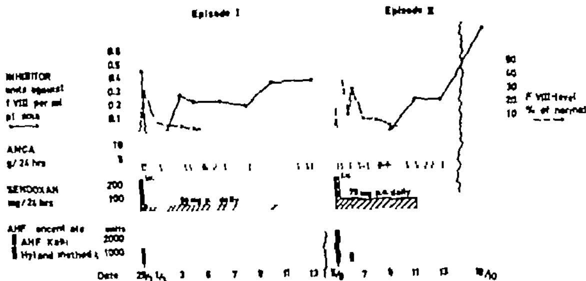


Fig. 2. Case 2 (8 years, weight 20 kg, severe haemophilia A). Course and treatment in first and second bleeding episode.

to raise the content to 25–30% of normal. The patient had no anticoagulant, as judged from the simple anticoagulant test, but the quantitative inhibitor test revealed low anticoagulant titre (1 ml of the patient's plasma inactivated 0.4 U factor VIII). He was thereafter given AHF concentrates only in the control of heavy bleedings. After infusion of 3–4 bottles of AHF Kabi the inhibitor level fell from about 0.3 to 0.1 U/ml plasma, but afterwards rose in the following 2 weeks to reach level of 1.4–1.6 U/ml plasma. It then took months until the inhibitor again reached its original level. The concentration of AHF-related protein was 130%.

Treatment with factor VIII concentrate combined with cyclophosphamide (Fig. 1). In July 1972 the patient was admitted to hospital because of a massive bleeding in the left knee and extreme pain. The inhibitor level was 0.5 U/ml plasma. The patient was given factor VIII i.v. in a dose of 5 000 U (Hyland method 4) and 750 mg cyclophosphamide (Sendoxan®). The knee joint was punctured and 50 ml blood was withdrawn. The bleeding and the pain promptly stopped. A further 3 000 U factor VIII were given after 18 hours. No adverse reaction occurred. Oral Sendoxan® 150 mg a day was given on the following 10 days. After the first infusion the factor VIII level rose to 50%. 24 hours after the second infusion it was 15%, after which it fell rather slowly. After 4 weeks the anticoagulant reappeared, but only in low concentration. He made a rapid recovery from this joint bleeding and the mobility of the joint was the same as before.

Case 2

A boy born in 1964, with severe haemophilia A. Ever since one year of age he had had recurrent haemarthrosis of the knee, ankle and elbow joints and several large intramuscular haematomas. Between 1965 and 1970 he had received 30 plasma transfusions and 60 bottles of AHF Kabi at his local hospital. When first seen in Malmö in May 1970 the AHF level was 0.1%. After infusion of 900 U factor VIII (AHF Kabi), factor VIII increased only to 5% (his weight 17 kg). An anticoagulant against factor VIII corresponding to 0.4–0.5 inhibitor U/ml plasma was demonstrated. In May 1970 and Dec. 1971 he received 1 200/U factor VIII on 4 occasions because of heavy painful joint bleeding. The therapeutic response was poor and extension of both knees became permanently defective. At reexamination 2–8 weeks after the end of treatment the anticoagulant titre had risen to 1–2 U/ml plasma. The concentration of AHF-related protein was 103%.

Treatment with factor VIII concentrate combined with cyclophosphamide (Fig. 2).

Episode I (2/72). In Feb. 1972 the patient was admitted to hospital because of extremely painful massive bleeding in the left knee joint. The inhibitor level was 0.5 U/ml plasma. He weighed 18 kg. He was given factor VIII (AHF Kabi)

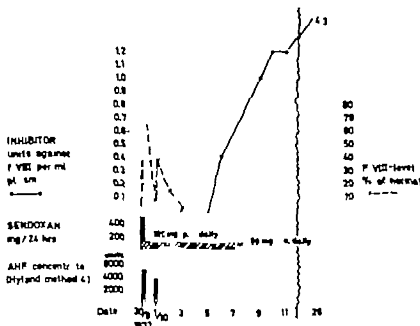


Fig. 3. Case 3 (14 years, weight 39 kg, severe haemophilia A). Course and treatment.

in a total dose of 1 200 U and 240 mg Sendoxan®. The knee joint was punctured and the pain abated. Bleeding stopped. Oral Sendoxan®) mg a day was given on the following 10 days. addition he received 3 g AMCA (Cyklokapron® Kabi) orally per day. No adverse reactions occurred. After infusion the AHF level rose to 30% and afterwards fell rather slowly. The inhibitor reappeared already on the third day but no secondary rise in inhibitor level was observed.

Episode II (9/72). In Sept. 1972 the patient was again admitted to hospital because of massive bleeding in the right knee joint. The anticoagulant titre was 0.7 U/ml plasma and his weight was 20 kg. He was now given 2 400 U factor VIII (Hyland method 4) and 300 mg Sendoxan® intravenously. The following day 800 U factor VIII were given. He also received 75 mg Sendoxan® orally per day for 6 days, after which the drug was withdrawn because of leukopenia. The joint was punctured after the first infusion. The therapeutic effect of the treatment was good and the bleeding regressed rapidly. Subsequent physiotherapy resulted in considerable improvement of the defective extension of the joint. The factor VIII content initially rose to 50% (Fig. 2). The inhibitor reappeared 6 days later but only in low titre (0.2 U/ml plasma).

Case 3

A boy born in 1958, with severe haemophilia A. Since early childhood the patient had had recurrent haemarthrosis of the knee, ankle and elbow joints, which had resulted in impairment of joint function, especially of the knee joints. He also had several large intramuscular haematomas and repeated episodes of haematuria. In 1968 he had received 10 blood transfusions, 40 plasma transfusions and 36 bottles of AHF Kabi at his local hospital. When first seen in Malmö in Aug. 1968, the factor VIII level was 0.1% and he was found to have an anticoagulant against factor VIII in a concentration corresponding to 0.4 U/ml plasma. Infusion of 1 200 U factor VIII (AHF Kabi) raised the level of the latter only to 3%. The inhibitor increased to 12 U/ml plasma within a few days. In 1969–72 the inhibitor level ranged from 0.6 to 1.2 U/ml plasma. No further doses of AHF concentrates were given. The concentration of AHF-related protein was 120%.

Treatment with factor VIII concentrate combined with cyclophosphamide (Fig. 3). In Sept. 1972 the patient was admitted to hospital because of enormous bleeding in the right hand and forearm after a blow. His finger joints were distorted and sensibility in the entire area of the median nerve was reduced. The inhibitor level was 1 U/ml plasma. He was given AHF i.v. in a dose of 5 000 U (Hyland method 4) and 500 mg Sendoxan®. The following day he was given a further 3 400 U AHF and Sendoxan® by mouth in a dose of 125 mg/day for 8 days; the

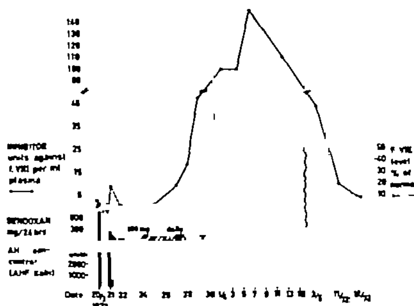


Fig. 4. Case 4 (42 years, weight 65 kg, sever haemophilia A). Course and treatment.

dose was then reduced to 50 mg/day because of leukopenia. The factor VIII level initially rose to 75% and then fell rather slowly. Bleeding promptly stopped. The inhibitor did not reappear until 5 days later but then in about the same concentration as before treatment.

Case 4

A man, born in 1930, with severe haemophilia A. Ever since early childhood he had had repeated haemarthrosis and large intramuscular haematomas. He had also had repeated episodes of gastrointestinal, renal and retroperitoneal haemorrhages. He had received at least 100 blood transfusions. When first seen in 1958 the factor VIII level was 0%. An anticoagulant against factor VIII in a titre of 1/5 was found. He was afterwards treated for long periods with prednisone, but without any demonstrable effect on the anticoagulant titre. During 1969 and 1970 the inhibitor level ranged between 1 and 3 U/ml plasma. The concentration of AHF-related protein was 104%. In connection with severe renal bleeding in one kidney—the other had ceased to function—and during 3 episodes with extensive intramuscular haematomas he received about 3 000–4 000 U AHF (AHF Kabi). This was not followed by any increase in factor VIII or neutralization of the inhibitor. After a few days the inhibitor level increased 5- to 10-fold.

Treatment with factor VIII concentrate combined with cyclophosphamide (Fig. 4). In March 1972 the patient was admitted to hospital because of severe bleeding in the left iliopsoas area with loss of sensibility and movement in the area supplied by the femoral nerve. Hb fell from 14 to 5.6 g/100 ml within 4 days. The patient was in a state of preshock because of the pain. The inhibitor level was 2.4 U/ml plasma. In the course of 2 days he received 1 000 mg Sendoxan® and 6 500 U AHF (AHF Kabi) intravenously. Since highly active AHF concentrate (Hyland method 4) was not available, it was not possible to give factor VIII in sufficiently large amounts because it would require infusion of such a large volume of fluid. On the 10 following days he was given 150 mg Sendoxan® by mouth a day. No adverse reactions were observed. Treatment had no notable effect on the bleeding. Factor VIII rose to at most 8%. The inhibitor level remained almost at zero for 4 days, but then increased markedly. After 10 days the inhibitor level was thus about 100 U/ml plasma.

Case 5

A previously healthy woman, born in 1909 developed signs of a severe bleeding disease in 1968—mainly in the form of spontaneous very large muscle haematomas. In 1968 a circulating anticoagulant against factor VIII was demonstrated in a titre of 1/10. After infusion of 2 000 U factor VIII (AHF Kabi) the anticoagulant titre rose to 1 100 within a few days. The patient was treated with prednisone for 3 months and later with Imurel® (100 mg by mouth a day) for 1 month, but without any demonstrable effect on the anticoagulant titre. No further infusions of factor VIII were given. In 1970 and 1971 the anticoagulant titre rose from 40 to 160 U/ml plasma. The concentration of AHF-related protein was 355%. The patient had originally had no

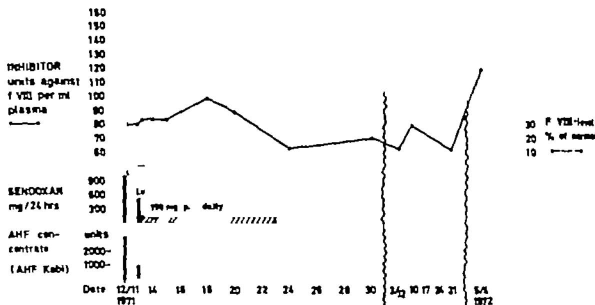


Fig. 5. Case 5 (63 years, weight 72 kg). Course and treatment.

signs of any other disease. In the last year however electrophoresis had revealed supervention of small M component and positive reaction in the determination ANF

Treatment with factor VIII concentrate combined with cyclophosphamide (Fig. 5). In Nov 1971 the patient was admitted to hospital because of bleeding in the region of the right thigh, which resulted in paresis and reduced sensibility of the right leg. Hb had fallen from 14.0 to 7.2 g/100 ml. The anticoagulant titre was 162 U/ml plasma (weight 71 kg). The patient was given 1 000 mg Sendoxan® intravenously and 3 000 U factor VIII (AHF Kabi). Owing to lack of a more active AHF preparation we refrained from giving factor VIII in larger quantities, because we were afraid of overloading the circulation. The following day the patient was given 500 mg Sendoxan® intravenously and it was intended that she should be given a large dose of factor VIII. But she reacted with chills and itching and the infusion was stopped after administration of 1 000 U factor VIII. On the following 10 days the patient received 150 mg Sendoxan® by mouth. No factor VIII activity could be demonstrated in the plasma (Fig. 5). The inhibitor level fell from 160 to 65 U/ml and no secondary rise of the inhibitor level occurred.

DISCUSSION

Four of the five cases with inhibitors against factor VIII described above had haemophilia A, while the fifth was a previously healthy woman in whom a spontaneous inhibitor against factor VIII developed. It is probable that the development of the inhibitor in this case was a link in a collagen disease. In all of the patients the content of AHF-related protein was normal or high, an observation according with what has previously been reported by Holmberg and Nilsson (9) and Prentice and Forbes (17). All the cases illustrate that substitution therapy alone is not sufficient in the management of patients with inhibitors. In cases 1, 2 and 3, in whom the inhibitor level was less than 1 U/ml plasma, it was admittedly possible to neutralize the inhibitor and raise the factor VIII level, but only for a few hours, which was followed by a marked increase of the inhibitor activity within 3–4 days.

As pointed out in the introduction, the use of chemotherapeutic agents and steroids in patients with haemophilia and inhibitors has been disappointing. In our case 4 prednisone had been given for long periods, but without any appreciable effect. The use of cytostatic agents has, on rare occasions, been accompanied by a decrease in the titre of factor VIII antibody in patients with

spontaneous inhibitors (23). Our patient 5 received prednisone for 3 months and Imurel® for 1 month but without any effect on the anticoagulant titre.

Robboy et al. (18) and Green (7) introduced a new form of treatment of patients with spontaneous inhibitors against factor VIII. It consisted of simultaneous administration of a large dose of factor VIII combined with immunosuppressive drugs. Robboy et al. (18) used azathioprine and prednisone and Green (7) used cyclophosphamide. In their two cases bleeding quickly stopped and the antibody promptly disappeared. Factor VIII was normal during the 5 and 7 months of follow-up respectively. Nilsson et al. (16) recently tried this type of treatment on four occasions in two patients with severe haemophilia B complicated by inhibitors of factor IX. A large single dose of factor IX concentrate and cyclophosphamide resulted in a prompt decline in antibody titre. It was apparent that suppression of antibody formation required administration of factor IX in doses large enough to neutralize the inhibitor and at the same time to raise factor IX to at least 50%. The inhibitor level remained at zero for 12 days to 3 months, after which it began gradually to rise to its original level. There are only few reports on the use of this combined treatment of haemophilia A complicated by inhibitors. Lusher et al. (12) prevented the secondary rise in antibody titre in two boys with haemophilia A and inhibitor by administration of cyclophosphamide combined with cryoprecipitate, but they did not give such large doses of factor VIII as Green (7) and Robboy et al. (18). Lechner et al (11) did not succeed in preventing the increase in inhibitor titre in two patients with haemophilia A and inhibitor by giving azathioprine simultaneously with factor VIII concentrate.

It is apparent from our investigation that a large dose of antigen (factor VIII) combined with cyclophosphamide may also be effective in haemophilia A with inhibitors, provided it is possible to give factor VIII in doses large enough to neutralize the inhibitor and to raise the factor VIII level sufficiently to secure haemostasis, i.e. to factor VIII levels of 40–60%. In our cases 1 2 and 3 with inhibitor levels of less than 1 U/ml plasma, such treatment had a satisfactory haemostatic effect. In case 1 the inhibitor level remained at zero for 10 days, but in the other two cases only for 3–5 days, after which it began gradually to rise toward its original level. In case 4 with a higher inhibitor level, namely 2.5 U/ml plasma, we never succeeded in raising the factor VIII level to more than 5–8%. In that case a 20-fold increase of the inhibitor activity occurred within 8 days, and after 14 days the inhibitor level had risen to as high as 140 U/ml plasma. Neutralization of the inhibitor and subsequent increase of factor VIII content to about 50% in this case would ha e required about 10 000 U factor VIII. If highly concentrated AHF preparation had been available, it might have been possible to control the bleeding also in this case. In case 5 the non-haemophilic woman with an acquired inhibitor against factor VIII, the inhibitor level was very high, namely 160 U/ml plasma. Neutralization of the inhibitor and increase of the factor VIII to about 50% in this patient would have required about 480 000 U We nevertheless thought it might be interesting to find out how the patient's antibody titre reacted to AHF concentrate combined with relatively large doses of antigen and Sendoxan® This combined treatment suppressed the inhibitor level far below what would have been possible with the dose of factor VIII alone, besides which no secondary rise occurred in the inhibitor level. In a case like this, infusion of sufficient factor VIII might require preceding exchange transfusions by plasmapheresis in order to decrease the inhibitor level.

It is obvious that in patients with haemophilia A with inhibitors the use of AHF preparation in doses large enough to raise the AHF content to about 50% combined with Sendoxan® will result in satisfactory haemostasis with the disappearance of the anticoagulant for 5–10 days and also which is very important, prevent the secondary increase in the inhibitors. In the treatment of patients with inhibitors in values exceeding 2 U/ml plasma, access to highly active AHF preparations is absolutely necessary On the other hand, in haemophilia A it does not appear possible to secure a constant disappearance of the anticoagulant, as described by Green (7) and Robboy et al. (18) in their non-haemophilic patients with acquired inhibitors against factor VIII. Neither in haemophilia B did Nilsson et al. (16) find a large dose of factor IX and cyclophos-

Table I. *Results of coagulation studies*

	Feb. 18	March 3	7	10	17	24	April 5	May 3	Normal range
Coagulation time (min)									
Glass	15	> 60	> 60	> 60	45	35	7	7	6–14
Plastic	30	> 60	> 60	> 60	90	80	22	22	12 32
Platelets/mm³	60 000	242 000	222 000	204 000	292 000	364 000	206 000	404 000	200 000–400 000
Bleeding time (Duke) (min)	3	5							
One-stage prothrombin time (sec)	15	*84*	*120*	*84*			14	15	14–16
P & P ( )	73	49	65	76			102	124	80–120
Factor V ( )	100	*0*	*0*	*0*	*0*	*0*	25	95	80–120
Factor VIII ( )		275		330	250				
Fibrinogen (g/100 ml)	0.28	0.58	0 91	0.85			0 51	0.35	0 20–0 40
FDP in serum (µg/ml)	0	25	30	40	0	15	0	0	0–5
Plasminogen ( )	25	90	90	75			145	110	60–140
α_2-macroglobulin ( )		76	88	87			121	103	80–120
Fibrinolytic activity on resusp. euglob. prec. (lysed area in mm²)	0	0	0	0			48	71	0–70
Anticoagulant against factor V (no. of U of f V inactivated by 1 ml plasma)	0	*29*	*31*	*23*	*17*	*13*	*0*	0	0
Antithrombin III ( )									
Clotting method	94	118					120		75–120
Immunochem. method	100	105					100		75–120

Inhibitory activity against factor V. One part of normal plasma was incubated with (*a*) 1 part of barbital buffer (*b*) 1 part of patient's plasma for 30 min at 37°C. After incubation the mixtures were assayed for residual factor V activity according to the method described above. The inhibitory activity of the plasma was expressed as the number of units of factor V inactivated by 1 ml plasma (1 ml normal plasma is said to contain 1 U factor V).

CASE REPORT

The patient was 65-year-old man who had had progressive prostatism for one year On admission to hospital in Dec. 1971 L urography suggested renal tumour which was confirmed by renal angiography No demonstrable metastases in the liver lungs or in the skeleton. Hb, serum creatinine and ESR were normal. Ten days after admission the left kidney was excised and 2 months later the prostate.

Postoperatively after the prostatectomy the patient bled heavily from the operation wound, and was given 1 500 ml blood, 1 000 ml Macrodex® and 400 ml plasma, AMCA and vitamin K. During the 5-hour operation the systolic BP never fell below 100 mmHg. On the day after the operation (Feb. 18) coagulation studies showed a low platelet count and normal P&P and factor V levels. The plasminogen level was low (Table I)

Two days after the operation the patient had chills, high grade fever and went into shock. He was treated i. with large doses of steroids combined with cephalothin (Keflin®), 1 g 4 times a day i. Blood cultures as well as urine cultures gave a significant growth of Klebsiella aerogenes. During the following days the platelet count decreased, the serum creatinine and urea increased, and peritoneal dialysis was started because of threatening high potassium level (6 6 mEq/l). Because of increasing temperature cloxacillin i was given together with cephalothin. The patient was treated with haemodialysis, but he required only remarkably small doses of heparin (2 000 NIH units/dialysis). He developed exanthema round the mouth and on the abdomen. Two weeks after the operation (March 3) he started to bleed profusely from the nose, the throat and the gastrointestinal and urinary tracts, and fresh blood was given. Coagulation studies revealed that he had developed an anticoagulant directed against factor V. On the 7th of March 500 mg cyclophosphamide (Sendoxan®) was given i. and on the following 10 days 100 mg cyclophosphamide per os a day. He received 2 transfusions of washed blood cells. The cephalothin and cloxacillin therapy was withdrawn (Fig. 1).

The bleedings stopped. After another 5 periods of haemodialysis the production of urine increased and the serum creatinine fell spontaneously. The patient left hospital 10 weeks after the operation with a serum creatinine of 2.7 mg/100 ml and creatinine clearance of 43 ml/min. During subsequent follow-up once a month his blood and coagulation status were normal and, when he was last seen 6 months after the operation, renal function was normal.

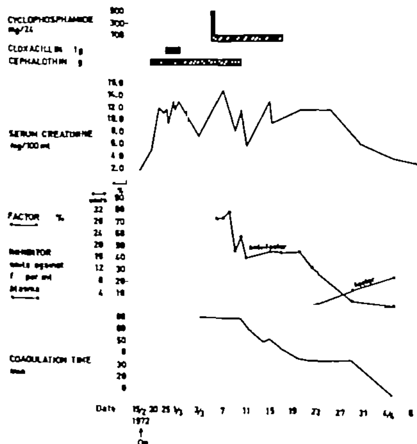


Fig. 1 The course of the disease.

RESULTS

Coagulation studies

The results of the coagulation and fibrinolytic studies during the patient's disease are summarized in Table I. On the 18th of Feb., during the bleeding episode in connection with the prostatectomy the platelet count was low (60 000/mm³), but P&P and factor V were normal. The plasminogen level (25%) was, however low and indicated a previous episode of fibrinolysis. On the 3rd of March the coagulation times in glass and plastic tubes and the one-stage prothrombin time were markedly prolonged. P&P was 49% and factor V 0%. No signs of fibrinolysis. Negative ethanol gelation test. The thrombin time was normal. The patient's plasma in dilution 1/10 prolonged the recalcification and one-stage prothrombin times of normal plasma. Addition of normal plasma failed to normalize the prolonged coagulation times. When the inhibitory activity against factor V was tested, it was found that the patient's plasma inactivated the factor V activity in normal plasma (29 U factor V neutralized by 1 ml of patient's plasma). On the 7th of March therapy with Sendoxan® was started and on the next day antibiotics were withdrawn, it being suspected that they might have been responsible for the appearance of the anticoagulant. The anticoagulant activity as well as the level of factor V were followed daily (Fig. 1). During the treatment with Sendoxan® the anticoagulant activity successively diminished, and on the 5th of April no anticoagulant could be demonstrated. Factor V began to rise and on the 10th of April the level was normal (110%).

Specificity of the anticoagulant

When normal plasma was incubated with the patient's plasma, only factor V activity was significantly decreased and the remaining coagulation factors (factors VIII, IX, XI+XII, P&P)

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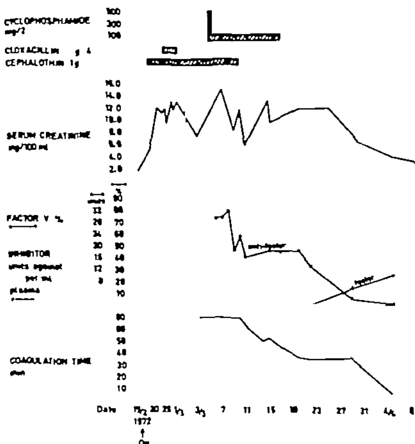


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Specificity of the anticoagulant

When normal plasma was incubated with the patient's plasma, only factor V activity was significantly decreased and the remaining coagulation factors (factors VIII, IX, XI+XII, P&P)

remained unchanged indicating that the anticoagulant is specifically directed against factor V

The anticoagulant was found to be neutralized by antihuman IgG antiserum when tested according to Feinstein et al. (11). The patient had normal γ-globulin levels (IgG 1 0 IgA 0.25 IgM 0.05 g/100 ml).

Physicochemical properties of the anticoagulant

The anticoagulant concentration was equally high in serum and in plasma and was not removed from plasma by adsorption with $BaSO_4$ or dialysis across a semipermeable membrane for 24 h. The activity was unaffected by heating at 56 C for 30 min.

DISCUSSION

The patient received multiple blood transfusions in connection with prostatectomy Four days afterwards he went into septic shock and treatment with cephalothin was started. After 12 days treatment with this antibiotic the patient started to bleed diffusely and coagulation studies showed a factor V level of 0% A circulating anticoagulant gainst factor V was demonstrated. The anti-gulant was neutralized by an antiserum inst IgG indicating that the anticoagulant onged to the IgG class.

Circulating anticoagulants against factor V have been regarded as a very rare cause of haemorrhagic diathesis. It has recently been suggested by Feinstein et al. (11) that the synthesis of immunoglobulins reacting with clotting factors is a major pathogenetic mechanism for acquired heamorrhagic disease in man. They also suspected that factor V anticoagulants are not as rare as past documented experience would suggest. The two patients with factor V anticoagulants described by Feinstein et al. were thought to have developed their antibodies as a consequence of multiple blood transfusions. Blecker and Williams (4) described a patient with an anticoagulant against factor V who had a history of penicillin allergy The patient reported by Lopez et al. (20) was also allergic to penicillin. As pointed out in the introduction, there are several cases in which factor VIII inhibitors developed in association with penicillin allergy (14). The patient observed by us developed the factor V anticoagulant after 12 days treatment with cephalothin. He had, however also received multiple blood transfusions, which have been regarded as one cause of the development of anticoagulants. The fact that inhibition of factor V by cephalothin and cephalonidin has been found in vitro by Raccuglia and Waterman (29) suggests that this therapy was the most likely cause of the development of the anticoagulant in our patient. Yudis et al. (32) recently described an anuric patient who developed a haemorrhagic diathesis and a prolonged prothrombin time during treatment with carburicillin, which further stresses the ability of penicillin to induce anticoagulants against different clotting factors.

The treatment of patients with anticoagulants has created many problems. Steroids have been used with poor results (14 21) except in occasional cases (28). One of the patients described by Feinstein et al. (11) was treated with ACTH with good effect. Immunosuppressive therapy has been used in recent years in the treatment of anticoagulants against factor VIII with some effect (30). A combination of cyclophosphamide and high dose of the antigen, factor VIII, has been used with success by Green (15) in the treatment of a woman with an acquired anticoagulant against factor VIII. Nilsson et al. (26) used the same treatment in two patients with severe haemophilia B and high titres of anticoagulants against factor IX, with excellent result. In these patients the anticoagulant disappeared during the treatment, but reappeared after 3 weeks and 6 months, respectively Also chlorambucil has been reported to have a good effect on anticoagulants against factor VIII (23) Our patient with a high titre of anticoagulant against factor V was treated with cyclophosphamide in a large initial dose and then in a smaller one for 10 days. Three days after the onset of this treatment the anticoagulant titre began to decrease and had disappeared 1 month after the beginning of treatment and the factor V concentration had begun to increase and then reached normal level. It is, of course not possible to exclude the possibility of a spontaneous disappearance of the anticoagulant after discontinuation of the cephalothin therapy The prompt disappearance of the anticoagulant suggests that immunosuppressive therapy is beneficial. In the earlier published cases treated with inhibitors against factor V the inhibitors were transient, but they nevertheless lasted for at least 2 weeks.

Judging from our experience the possibility of anticoagulants developing during penicillin or cephalothin therapy must be borne in mind when patients receiving such treatment show an increased bleeding tendency. Such anticoagulants may be successfully treated with cyclophosphamide.

ACKNOWLEDGEMENT

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COAGULATION STUDIES IN CHRONICALLY BEDRIDDEN PATIENTS

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Abstract. Twelve patients without clinically recognized venous thromboembolic disease have been studied. In none of them could obvious signs of hypercoagulability or intravascular coagulation be demonstrated. The clinical implications of these findings are briefly discussed.

Stasis of blood, hypercoagulability and lesions of the vessel wall are considered to be the main pathogenetic factors of deep vein thrombosis, as originally postulated by Virchow (15). Bed rest is accompanied by venous stasis and is associated with a high incidence of phlebothrombosis (18). Further a direct relationship between duration of bed rest and frequency of venous thrombosis has been reported (3). On the other hand, chronically bedridden patients have a strikingly low incidence f thrombotic disease (11).

The aim of the present study was to see whether signs of hypercoagulability or of intravascular coagulation could be demonstrated in patients chronically confined to bed but without serious somatic illness and without clinically recognized thromboembolic complications.

MATERIAL AND METHODS

T elve patients, aged 67–97 years, ere studied. They all suffered from cerebral atherosclerosis with dementia of different degree, and had been confined to bed for 1 1/2 8 years. Eight f them were completely apathic and lay almost immobile in the bed. A plastic muscular rigidity of the extremities as common. T patients had significant edemas and ere treated with diuretics, one received digitoxin because f moderate heart failure, and one was treated ith insulin because of diabetes ith complications from different organs. Most of the patients ere at times given sedative and hypnotic drugs.

The blood analyses performed and references for description of the methods used are given in Table I.

RESULTS

Most values were within normal limits (Table I), and in no instance were definitely increased values found. Three patients had lowered factor V level (34 52 and 65 %). One patient exhibited a slightly lowered thrombocyte count (87 500/µl), whereas another had a lowered thrombocyte count (82 000/µl) and antithrombin III level at lower normal limit (97 %). In no patient was a positive ethanol test observed.

COMMENTS

In disease associated with high incidence of venous thrombosis, hypercoagulability of the blood, as indicated by high levels of fibrinogen, factors V and VIII and platelets are regularly found. In addition a positive ethanol test for soluble fibrin in plasma is frequently observed (10). In some patients this condition is transformed into disseminated intravascular coagulation, as reflected by decreasing values of the above mentioned factors together with a positive ethanol test. In addition, a low antithrombin III level in plasma is observed, due to consumption caused by intravascular thrombin formation (1).

In the present study sligthly lowered values of factor V antithrombin III and thrombocytes were occasionally seen. In no instance, however was a positive ethanol test found As this test seems to be a sensitive indicator of soluble fibrin in plasma (9), the occurrence of even low graded intravascular coagulation in our patients may probably be ruled out.

Our findings suggest that slowing of the circulation due to bed rest as the only precipitating factor is inadequate to induce cli

Table I *Coagulation values in the 12 patients studied*

	Reference	Normal values	Patients' values	
			Mean	Range
Platelets ($10^3/\mu l$)	(4)	100–400	141	82 225
Normotest (%)	(13)	65–150	90	76–110
Thrombotest ( )	(12)	> 50	70.5	50–100
Thromboplastin time (sec)	(16)	13–17	17	14.5–19
Partial thromboplastin time (sec)	(6)	60–85	75	70.5–83
Fibrinogen (mg)	(5)	170–400	323	235–470
Antithrombin III (plasma) ( )	(8)	100–150	118	90–145
Factor V ( )	(14)	70–120	85	34–110
Thrombin time (sec)	(7)	20–23	22	19–25
Ethanol test	(10)	Neg.		Neg.

cant thrombosis or signs of consumption coagulopathy In accordance therewith the survival of autologous platelets in healthy subjects is not affected by bed rest (2), and repeated animal experiments have shown that stasis alone fails to induce blood clotting (17). To initiate thrombogenesis, therefore, additional factors, such as percoagulability are probably required.

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URINARY FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS (FDP) AND GLOMERULONEPHRITIS

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Abstract. In concentrated 24-hour urine from 24 patients with incipient glomerulonephritis without uraemia the fibrin/fibrinogen degradation products (FDP) have been studied. In 18 of 19 patients with proliferative glomerulonephritis high molecular weight degradation products (HMWDP) were found, while one patient had D and E products probably due to further degradation of the HMWDP in the lower urinary tract. Only 2 patients of 5 with membranous glomerulonephritis had HMWDP in the urine in low concentrations. All patients with proliferative glomerulonephritis had selective proteinuria with an excretion predominantly of albumin and transferrin, which makes it improbable that the HMWDP found in the urine from these patients are derived from filtration through the glomeruli. Thus the urinary HMWDP found in these patients most probably originate from lysis of fibrin deposits in the kidney and are excreted in the urine. It is concluded that the FDP may be demonstrated in the urine also in incipient glomerulonephritis provided that the urine is concentrated. These FDP most probably originate from the kidneys.

Intraglomerular fibrin deposits have been found along the basement membrane or intramembranously in different forms of glomerulonephritis, especially the proliferative types (4, 8, 9, 10, 17). The granular or linear subepithelial deposits found in membranous glomerulonephritis have been shown to contain IgG (9), while the presence of fibrin has been doubted in this form of glomerulonephritis.

The presence of fibrin/fibrinogen degradation products (FDP) in the urine has been described as an indirect but reliable sign of fibrin deposits in the kidneys (8, 10, 11, 18, 1, 25, 27, 28). However most patients in earlier studies were uraemic when examined, so that the renal damage must have been extensive. In order to find out whether the urine contains FDP in early renal disease, we studied 24 patients with early glomerulonephritis which had not produced severe renal insufficiency. For the evaluation of the origin of the FDP occurring in the urine from patients with renal diseases it is important to know what type(s) these FDP are. The FDP found in concentrated 24-hour urine volumes in the patients included in this study, therefore, were typed by the method of Bouma et al. (5).

CLINICAL MATERIAL

Twenty-four patients with various forms of glomerulonephritis were studied. The diagnosis was made on the basis of clinical, haematological and biochemical findings and on the light microscopic appearance of fine needle aspiration biopsy specimens of the kidney. The serum creatinine clearance and the endogenous 24-hour creatinine clearance were normal in all of the patients.

The renal disease was classified according to Brun (personal communication). The cases were classified histologically as follows: 1) focal and generalized proliferative glomerulonephritis (12 pats.), 2) focal segmental sclerosing glomerulonephritis (6 pats.), 3) systemic lupus erythematosus (1 pat.), 4) membranoproliferative glomerulonephritis (1 pat.), 5) epimembranous glomerulonephritis (2 pats.), 6) extracapillary proliferative glomerulonephritis (1 pat.) and 7) lobular glomerulonephritis (1 pat.)

METHODS

Collection of urine. Twenty-four-hour specimens were collected without addition of any fibrinolytic inhibitor. The urine was concentrated 20–1 000 times against a membrane that retained proteins with a mol.wt. of more than 12 000 (Diaflo, Amicon Corporation, Massachusetts). FDP and protein in the urine (a Biuret method) were determined before and after concentration of the sample.

Determination of FDP. These products were deter

Table I. *Findings in serum (s) and urine (u) from 24 patients with different types of glomerulonephritis*

Type of glomerulonephritis	Case no.	FDP/s (µg/ml)	FDP/u (µg/ml)	FDP/u (µg/24 h)	Type of FDP	Urokinase act./u (Ploug U/ml)	Albumin/ (g/l)	Plasminogen/u ( )	α_2-macroglobulin/u ( )
Focal and generalized proliferath	1	0	0	875	HMW	6	1.3	—	—
	2	0	0	350	D+E	11	<0.05	—	—
	3	0	0	175	HMW	3	1.9	—	—
	4	0	0	560	HMW	4	0.5	—	—
	5	0	0	0	—	2	<0.05	—	—
	6	0	0	80	HMW	3	<0.05	0	—
	7	0	0	234	HMW+D	1	0.1	0	0
	8	0	0	22	HMW	2	0.05	0	●
	9	0	0	150	HMW+E	1	0.2	0	0
	10	0	0	50	—	1	<0.05	0	●
	11	0	0	161	—	—	<0.05	0	●
	12	0	0	300	HMW	2	<0.05	—	—
Focal segmental sclerosing	13	0	0	90	—	6	0.2	—	—
	14	0	0	300	HMW+E	—	0.4	—	0
	15	0	0	120	—	1	0.7	0	●
	16	0	0	68	HMW	4	0.1	0	●
	17	0	0	1650	—	1	2.7	0	—
	18	0	0	48	—	2	0.1	0	●
SLE	19	0	0	300	HMW	2	0.5	0	0
Membranoproliferath	20	0	0	68	HMW	1	2.1	0	0
Epimembranous	21	0	0	30	HMW	3	2.3	0	0
	22	0	0	0	—	3	5.2	0	●
Extracapillary proliferative	23	0	0	0	—	—	<0.05	0	0
Lobular	24	0	35	—	HMW	2	4.0	—	—
Controls (=19)		0	0	0	—	>8	<0.05	0	0

—d in the serum and in the urine by the immunological method of Nilehn (24) using a specific antiserum against the D product in the rocket method of Laurell (19). The urine samples (dil. 1 1) were applied to agarose plates (1% agarose) containing the anti-D-serum. On high voltage electrophoresis the antigen is forced into the gel. Precipitation peaks form and their heights ar proportional to the amount of antigen in the sample. High molecular weight fibrin/fibrinogen degradation products (HMWDP) are used as standard. This method demonstrated no FDP in unconcentrated urine from 200 healthy controls (13). Neither were any FDP found in concentrated 4-hour specimens from 19 controls.

Typing of FDP in the concentrated urine was performed according to Bouma et al. (5) According to this method urine samples (dil 1 1) are applied to separate agarose gel plates containing antisera gainst human fibrinogen, D and E fractions, espectively Human fibrinogen is used as standard for all the plates. The type(s) of FDP and their concentrations are calculated from the single or double electrophoretic peaks produced by the reaction between the urine and the antiserum. Addition of a substance identical with that present in the sample results in an increase in the height of the peak. If the substance added is not identical, the result will be a double peak. The HMWDP were prepared essentially according to Marder et al. (20) with a plasmin digestion of fibrinogen for 10 min followed by gel filtration on Sephadex G-200 (Pharmacia, Uppsala, Sweden, phosphate buffer 0.01 M, pH 7.5). The D and E products were prepared essentially according to Nilehn (23). In the preparation of the E product the fibrinogen digest was heated (56°C for 30 min) to denature the D product before chromatography on DEAE cellulose (Serva Entwicklungslaboratorium, Heidelberg, Germany) in the same way as for the D product.

α_2-macroglobulin, plasminogen and albumin were measured in the concentrated urine immunochemically with specific antisera in the rocket method of Laurell (19). No α_2-macroglobulin or plasminogen could be demonstrated in the concentrated urine from the 19 controls. The albumin concentration was found to be below 0.05 g/l.

The urokinase activity was determined in unconcentrated urine by Andersson clot method (1). The urokinase in diluted urine (1/5) activates added plasminogen and the plasmin formed is lowed to lyse a clot clot formed from bovine fibrinogen and thrombin. The time for complete lysis is taken as a measure of the urokinase activity. In 10 control persons w obtained values abov 8 Ploug U/ml.

Serum and urine creatinine concentrations were measured with Technicon AutoAnalyzer by standard techniques by the Department of Clinical Chemistry and the *creatinine clearance* was calculated.

Electrophoresis was performed on the urine (Department of Clinical Chemistry) to determine hether the

proteinuria was of selective (excretion of predominantly albumin and transferrin) or an unselective type (excretion of also high molecular weight proteins such as γ-globulins).

RESULTS

Proliferative glomerulonephritis. No FDP could be demonstrated in the serum or unconcentrated urine from the 19 patients with different forms of proliferative glomerulonephritis. Such products were, however found in amounts up to 1 650 μg/24 h in concentrated 24-hour urine specimens from 18 of the 19 proliferative glomerulonephritis patients. One patient had no FDP in the concentrated urine specimen either and the concentration of albumin was also fairly low (<0.05 g/l).

The FDP in the urine from 12 of the patients were typed. Eleven of them had HMWDP the 12th only D and E products. In that patient the urokinase activity in the urine was high (11 Ploug U/ml) while in all the others it was rather low (1–6 Ploug U/ml).

The albumin concentrations ranged from <0 05 to 2.7 g/l. None of the patients had plasminogen or α_2-macroglobulin in the urine (Table I)

Membranous glomerulonephritis. None of the 5 patients with membranous glomerulonephritis had FDP in the serum. Such products were found in unconcentrated urine from one of them (35 μg/ml). Two of the other patients who had no demonstrable FDP in unconcentrated urine had FDP (68 and 30 μg/24 h) in the concentrated 24-hour specimen, while two had no FDP in the concentrated urine specimen either The FDP in the urine from all the patients who had FDP were found to be HMWDP

The urokinase activity of the urine varied between 1–3 Ploug U/ml. The albumin excretion ranged from <0.05 to 5.2 g/l. The patient with the highest concentration of albumin in the urine had no FDP None of the patients had plasminogen or α_2-macroglobulin in the urine. (Table I.)

The 19 patients with proliferative glomerulonephritis had proteinuria of selective glomerular type with excretion of predominantly albumin and transferrin, but no immunoglobulins, while the 5 patients with a membranous type of glomerulonephritis had unselective proteinuria with excretion also of high molecular weight proteins such as γ-globulins.

DISCUSSION

The amount of FDP excreted in the urine has been shown to vary with the activity and severity of the disease (10, 18, 25 27). The excretion of FDP in the urine has also been found to correlate with the histological picture and the extent of intraglomerular fibrin deposits (10). Only one of the 24 examined patients with glomerulonephritis of different types had demonstrable FDP in unconcentrated urine, indicating that the renal damage in 23 was not severe enough to result in the formation of FDP in amounts detectable by the method used. In our material FDP were demonstrable in concentrated 24-hour urine specimens from 18 of the 19 patients with proliferative types of glomerulonephritis and in 3 of 5 with membranous glomerulonephritis. This shows that FDP are excreted in the urine already in an early phase of the glomerular disease before any FDP can be detected in the serum. For the detection of the FDP in these patients the determination, however must be made in concentrated 24-hour urine specimens. Stiehm et al. (27) studied a few patients without azotaemia. They found FDP in concentrated 24-hour urine specimens only from 5 of 11 patients. They used a tube precipitin method and the difference may thus depend on the various methods used

No unanimity has been achieved concerning the type(s) of FDP found in the urine in renal disease. Some a thors have thus found low molecular weight products (6, 28, 30) by qualitative methods. Others, however have found only one sort of FDP in the urine, which has been interpreted as a high molecular weight fragment (2, 14 26). Recently Clarkson et al. (10), using gel filtration on Sephadex G-200 found predominantly D and E products in the urine from a few patients with proliferative glomerulonephritis and only small amounts of HMWDP But they did not mention whether the urine had been collected in the presence of an inhibitor of fibrinolysis. The findings of low molecular fragments may thus, perhaps be explained by the assumption that the urokinase activity was normal in the specimens and that the FDP had been degraded lower down the urinary tract. Using a specific immunochemical method Bouma et al. (5) found only HMWDP in the urine from uraemic patients, but only D and E products in urine from patients receiving thrombolytic therapy

Using the same method we found high molecular weight products in all the non-uraemic patients in this material except one with a proliferative type of glomerulonephritis. This patient had D and E products and proved to have a normal level of the urokinase activity of the urine, which is unusual in renal disease (7 29). In all the other patients in our material the urokinase activity was decreased. Such a decrease may thus be regarded as an early sign of renal disease. Since the urine had been collected without addition of a fibrinolytic inhibitor the finding of D and E products in this case may be explained by further degradation of HMWDP in the lower urinary tract.

In this material of non-uraemic patients we thus found HMWDP in concentrated urine. In normal kidneys HMWDP is not cleared through the glomeruli (5 26). All the patients with proliferative glomerulonephritis had selective proteinuria indicating that the disease was in an early phase. None of the patients had plasminogen in the urine either. The patients with membranous glomerulonephritis had unselective proteinuria, indicating more severe damage of the glomeruli. *Yet the levels of HMWDP in the urine were* low indicating a much smaller amount of in deposits in the kidneys in this form of glomerulonephritis, which is also in accordance with findings in earlier histological studies (10)

Judging from our results, it is possible to demonstrate the presence of a renal disease with fibrin deposits in an early phase by determining and typing the urinary FDP in a 24-hour urine specimen. The findings of high molecular weight degradation fragments in the urine from these patients in an early phase of their renal disease with a normal creatinine clearance suggest that *the FDP really originate from lysis of fibrin* deposits in the kidneys, at least in proliferative glomerulonephritis. Early determination of FDP in the urine must be useful when deciding whether treatment for dissolving or preventing the formation of intraglomerular fibrin clots is indicated or not. Such treatment has been recommended by many authors (3 15 16 ?7), although the pathogenetic importance and the extent to which the clinical picture of the renal diseases depends on the intraglomerular fibrin deposits is still obscure. When indicated, however such treatment should be started in an early phase of the disease. In addition, determination of FDP in the urine must be of great value in following the effect of the treatment used, both anticoagulant and other form of therapy such as immunosuppressive treatment.

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EOSINOPHILIC LEUKAEMIA —RECOVERY OF MYCOPLASMA ORALE FROM THE BONE MARROW

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Abstract. A case of eosinophilic leukaemia of the mature cell type is presented. The case reported fulfils the clinical and morphological criteria of acute myeloid leukaemia. The morphological and serological findings are not consistent with disseminated eosinophilic collagen disease or any other collagen disease. The onset was that of an infection with an elevated titre of cold agglutinins. *Mycoplasma orale* type 1 was isolated from the bone marrow. The possible oncogenic role of mycoplasma is discussed, inasmuch as the morphological defect found in the present patient's thymus may indicate potentially altered immune response.

Eosinophilic leukaemia (EL) is a rare type of myeloid leukaemia (ML). Its existence as a distinct entity and a malignant neoplasm of the haematopoietic tissue is no longer disputed (3, 4, 6, 7, 9, 10), although dissenters have considered EL to be a variant of disseminated eosinophilic collagen disease" (DECD) (2, 8, 17). Rickles and Miller (19) have recently discussed the relationship of EL to eosinophilic leukaemoid reaction with particular regard to the immunological aspects of eosinophilia. A case interpreted as EL is presented. Differential diagnostic considerations as well as the suggested aetiology will be discussed. The findings suggested a *Mycoplasma orale* infection from the beginning to the end of the clinical course.

CASE REPORT

Past history and heredity. A 3-year-old boy, born in Oct. 1963, the only child of healthy parents with no history of hereditary disorders, had been in good health except for otitis at the age of two. The WBC and eosinophil count of his parents were normal.

Course of disease (summarized in Table I)

The first acute stage began in Feb. 1967 with persistent febrile upper respiratory infection refractory to penicillin. Subsequent tetracycline therapy was interrupted because of a rash. Non-productive cough, dyspnoea, mild pain on movement and slight swelling of the knees were recorded.

On admission (March 8) physical findings disclosed generalized rash, and the liver and spleen were slightly palpable below the costal margin, but the general condition was good, with heart and lungs normal at auscultation and no enlarged lymph nodes. Signs of parasitic infestations common in the Finnish population were not found. The WBC was 79 000 cells/mm³ of which 72.5% were segmented eosinophils and 4.0% eosinophilic myelo- and metamyelocytes. The bone marrow showed an increase of myeloid cells, predominantly eosinophils, at various stages of maturation. Mononuclear cells, interpreted as paraleucoblasts, were also seen.

From March 13 onwards the child became progressively dyspnoeic, with severe cough and high temperature. Radiographically bilateral, miliary lung infiltrates, as seen in acute leukaemia, were found (Fig. 1).

Antileukaemic treatment was started with 6-mercaptopurine (6-MP) 50 mg and prednisone 50 mg daily. Within four days the pulmonary infiltrates disappeared (Fig. 2) and the general condition improved dramatically. No symptoms remained apart from the rash. No haematological remission was achieved however: leucocytosis, blood eosinophilia and the bone marrow changes, although without paraleucoblasts, persisted. With 6-MP the first partial remission lasted for five months. During this period the boy's general condition was good and he led a normal life.

The second acute stage started with cough and miliary infiltrates in the lungs. The liver and spleen were now markedly enlarged. Anaemia and thrombocytopenia occurred and the WBC count, dominated by eosinophils, increased. Paraleucoblasts were seen in the blood and the bone marrow. A biopsy from skin petechia showed non-specific arteritis-like inflammatory vascular reaction indistinguishable from that seen in diseases accompanied by eosinophilia. Treatment with 6-MP was discontinued. Prednisone 60 mg and methotrexate 5 mg daily per os were started. The latter drug was withdrawn because of thrombocytopenic bleeding. Oncovin was instituted (0.05 mg/kg). Dramatic restitution of the pulmonary infiltrates and the general condition was again achieved. The child was discharged subjectively well.

The second partial remission lasted only two weeks

Table I. *Main findings during the course of the disease since admission to hospital*

Month Stage of disease	March I acute stage	April–Aug. I partial remission	Sept. II acute stage	Oct. II partial remission	Nov. Terminal stage
General condition	Fair–poor good	Good	Poor	Good	Moribund
Red skin rash	+	+	+	+	+
Purpura	–	–	+	+	+
GI bleedings	–	–	–	–	+
Radio-opaque lung infiltrates	Miliary	None	Miliary	None	Bronchopneumonic followed by miliary
Palpable lymph nodes	–	–	+	+	+
Hepato- and splenomegaly	Slight	Slight	Marked	Marked	Marked
Skeletal X-ray (extremities, skull thorax)	Normal		Normal		
WBC count					
Cells/mm³	Max. 79 000	Min. 28 000	Max. 53 500	Min. 8 300	Max. 34 900
Eosinophils (%)	74.5	51.5	77.5	23	59.5
Paraleucoblasts in					
Blood	–	–	+?		+
Bone marrow	+?	–	+		+
Thrombocytopenia	–	–		+	+
γ-globulins (g/100 ml)	1.6	0.64	1.01		
Immunoelectrophoresis	Normal		IgM increased		IgM, IgG, IgA increased
Waaler Rose	–		64		–
Latex	– later		–		+ +
Antistreptolysin	Not elevated		Not elevated		Not elevated
Antistaphylolysin	Not elevated		Not elevated		Not elevated
Cold agglutinins	256		128		8
Coombs test	Negative		Negative		Negative
Antinuclear antibodies	Mouse liver		?		–
	Human leucocytes –		–?		–
cells oprecipitates	–		4		3%
Treatment	Prednisone 6-MP	6-MP	Prednisone Methotrexate Vincristine	Vincristine	Antibiotics Prednisone Vincristine

but the WBC fell to 8 300 cells/mm³ with 23% eosinophils. However the thrombocytopenia and anaemia were not corrected.

The terminal stage began with pneumonia, responding successfully to antibiotic therapy. Miliary pulmonary infiltrates and marked spleno-hepatomegaly developed. *Leucocytosis and eosinophilia rapidly increased*, 10% paraleucoblast-like mononuclear cells were found in the blood (Fig. 3). Myelocytes with few paraleucoblasts now dominated the bone marrow morphology. Severe thrombocytopenia ensued, causing multiple bleedings from the GI tract. Repeated transfusions were of no avail; without responding to treatment the boy died (Nov. 28) one month after the last admission.

Postmortem examinations

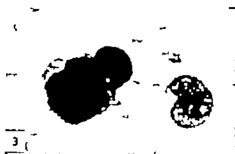
The boy showed moon face, oedema, and small and in places confluent skin petechiae all over.

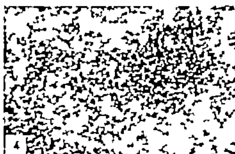
Recovered abnormal organ weights (g) (available normal values according to Lehd (16) within parentheses): heart 132.5 (71), brain 1 160 (900), right lung 326.8, left lung 234.6, kidneys 147.8 (100), liver 1 030 (495), spleen 457.7 (35).

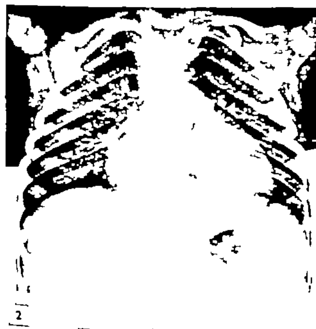
Macroscopic findings. Brownish, small miliary infiltrates in the lung parenchyma with brown, frothy bronchial mucus, yellowish pericardial effusion and epicardial petechiae with large haematoma in the right atrial wall and white miliary myocardial infiltrates. In addition to an anomalously short right ventricle; distally oesophageal ulcerations and blood in the GI tract; red marrow in the femoral diaphysis and subfascial bleedings in the femoral musculature were encountered. In the liver and the spleen the cut surface was blush and dense, in the kidneys pale.

Microscopic findings. The lungs showed subpleural, peribronchial, intra- and perivascular infiltrates consisting of immature, non-classifiable neoplastic blood cells and atypical eosinophils. The alveolar walls were thick and occasionally lined with hyaline membranes. Alveolar siderophages were frequently encountered in protein-rich exudate. No vascular lesions, typical of periarteritis nodosa or of any other defined arteritis, were











Figs 1 and 2. Lung infiltrates during the acute stage and the clearing effect of antileukaemic treatment.

Fig 3. Paraleucoblasts from the blood during the terminal stage.

Fig 4. Section from the liver (autopsy) showing diffuse leukaemic infiltrates.

Fig 5. Section from the myocardium (autopsy) showing only slight leukaemic infiltration. The vessels are filled with leukaemic cells.

seen in the lungs or in any other organ studied. The liver showed diffuse leukaemic infiltrates with increasing density in the portal zone (Fig. 4). The kidneys showed extensive typical leukaemic infiltrates. Microscopic infarcts were seen under the splenic capsule. The normal splenic parenchyma was distorted by packed masses of leukaemic cells as well as cells of the eosinophilic series. The normal lymph node architecture was destroyed by similar leukaemic infiltrates. No Reed-Sternberg cells or other features of Hodgkin's disease were detected. Only microscopic infiltrates were seen under the leptomeninges, but these were clearly leukaemic. Large epicardial leukaemic infiltrates with haemorrhages were found and also diffuse, streak-like leukaemic infiltrates of the myocardium (Fig. 5). The thymus did not contain any Hassall' corpuscles. Secondary changes attributable to antileukaemic therapy were seen. In all the organs studied the mast cell reaction was normal.

Studies on mycoplasm

A mycoplasma was isolated from bone marrow specimen taken at the beginning of the terminal stage (Oct. 23). The cultivation was performed using a modified mycoplasma medium as described earlier (12). The strain isolated did not convert to bacteria when penicillin and thallium acetate were omitted from the culture medium. It required sterol for growth. It did not ferment glucose or split urea, but metabolized arginine. Because the isolate did not form colonies visible to the naked eye, immune serum against it was prepared in rabbit. This was tested against mycoplasma species: M hominis, M. salivarium M orale type 1 M. fermentans, M arthritis, M. pulmonis and M. gallinarum. The immune serum inhibited the growth of M. orale type 1 with an inhibition zone of 3 mm. It did not inhibit the other mycoplasma mentioned. The growth inhibition test was performed according to Clyde (5).

DISCUSSION OF DIAGNOSIS

The following arguments in favour of EL in the present case may be advanced.

The clinical course of the case corresponded to that of acute leukaemia. The first and second acute phase responded promptly to antileukaemic treatment. After each relapse the subsequent periods of remission became successively shorter *ending in a terminal stage uncontrollable with* leukaemic drugs or fresh blood transfusions. [illegible]o- and hepatomegaly developed in the ini[illegible]stage, increasing throughout the disease. [illegible]ymphadenopathy occurred during the first relapse. Thrombocytopenia was first encountered during the first relapse and persisted throughout the disease. Severe thrombocytopenia and haemorrhagic diathesis occurred in the last relapse. The WBC morphology differed distinctly from that usually seen in acute ML. In the initial stage the bone marrow showed few and the blood no paraleucoblasts. Even terminally these cells were sparse both in the bone marrow and in the blood. With the exception of the second partial remission, marked leucocytosis persisted with eosinophilia exceeding 50%. The bone marrow WBC consisted predominantly of myelocytes, metamyelocytes, stabnuclear and polymorphonuclear cells of the eosinophilic series.

The main symptom in the acute stage was a severe cough, dyspnoea and miliary lung infiltrates. These symptoms and infiltrates disappeared entirely during antileukaemic treatment.

Autopsy revealed multiple eosinophilic infiltrates in various organs possessing no embryonic haematopoietic activity. All the organs studied microscopically showed leukaemic infiltrates. The histopathological picture presented above corresponds to that described in the literature (3).

Bentley et al. (3) have classified EL into two major types.

A) *Acute "blastic" EL*, with an increased number of blast cells in the blood and/or bone marrow from the onset. It should be mentioned that eosinophilic granula are not seen in the blast cell and the myeloid subclass has to be determined from the accompanying more mature cell types. B) *Mature cell EL*, presenting with only mature eosinophils, but the disease may either assume an acute course terminating in blast cell crisis or behave like a true chronic leukaemia with protracted clinical course.

Hauswaldt et al. (10) have summarized the diagnostic criteria of EL as follows: The disease must arise autochthonously and lead per se to death, terminating mostly but not always in a blast cell crisis. Eosinophils must constitute at least 50% of the WBC. Eosinophilic infiltrates should be found all over the body including tissues that have no embryonic haematopoietic activity.

Rickles and Miller (19) suggest more rigid criteria. Pronounced, persistent eosinophilia associated with immature forms, either in the peripheral blood or bone marrow. More than 5% blast forms in the bone marrow. Tissue infiltration by immature cells of predominantly eosinophilic type. An acute clinical course measured in months, accompanied by anaemia, thrombocytopenia, increased susceptibility to infection, and/or haemorrhage.

Although in its clinical course EL does not differ from ML, Bentley et al. (3) mention certain findings seen more frequently in this type of leukaemia. The dominant, as well as presenting, signs and symptoms are cough, transient radiological pulmonary infiltrates, rales and cyanosis, due to alveolar thickening secondary to pulmonary eosinophilia from any cause. Frequently overt heart failure occurs due to massive myocardial infiltrates with necrosis and mural thrombi. There may be extensive infiltration of the central nervous system. In cases of mature cell EL of acute and especially of chronic course, other non-malignant as well as malignant diseases with marked eosinophilia must be excluded.

In the early stages of mature cell EL the patient shows leucocytosis and extreme eosinophilia without immature cells.

DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

Benign diseases with eosinophilia are usually easily excluded. In eosinophilic granulomatosis of early childhood typical changes in the bone system are found.

Diseases with a malignant course and leukaemoid eosinophilia are periarteritis nodosa of the pulmonary type (hypersensitivity angiitis), acute parietal endocarditis with eosinophilia, some blood dyscrasias, Hodgkin's disease and malignant tumours (3). Acute parietal endocarditis with eosinophilia and fatal course is sometimes almost indistinguishable from EL, but at necropsy generalized leukaemic infiltrates are not found. Periarteritis nodosa of the pulmonary type may assume an identical, fatal course with, e.g., massive, multiple eosinophilic infiltrates. The vascular histopathological changes are, however diagnostic.

The existence of a separate disease entity called EL has been questioned by Odeberg (17) who suggests that EL and DECD may be variants of the same (auto-immune?) syndrome" DECD as defined by Engfeldt and Zetterström (8) is characterized by marked chronic or transient blood eosinophilia and involvement of many internal organs, especially the heart, lungs and the nervous system, as well as skeletal muscles, joints and skin. The histopathological lesions consist of focal interstitial infiltration and specific vascular lesions varying in severity from slight endarteritis to necrotizing arteritis. Under the heading of DECD the authors include known disease entities such as eosinophilic granulomatosis of early childhood, parietal endocarditis and eosinophilic myocarditis.

The histopathological manifestations of the present case are not consistent with the diagnosis of collagen disease as introduced by Klemperer et al. (15) and Pollack (18).

Primary disease of the connective tissue is excluded in the present case. The serological findings in this case do not qualify it for a diagnosis of autoimmune disease. Nor did we see the histopathological lesions of DECD described above.

The serological findings of the first acute stage showed a diffuse hypergammaglobulinaemia and cold agglutinins in a titre of 256. The latter finding was found in 84% of patients with *Mycoplasma pneumoniae* infection (13). *Mycoplasma orale* type I was isolated from the bone marrow specimen at the beginning of the final stage. No earlier cultures had been made. In the meanwhile the cold agglutinin titre had dropped to 8 However immunosuppressive therapy had been administered. Serum cryoprecipitates—found in 34% of patients with *Mycoplasma pneumoniae* infections (13)—were however encountered. Thus microbiological and serological data suggest a mycoplasma infection.

The concomitant *Mycoplasma oral* infection may be interpreted in three different ways A) The association is fortuitous. B) The infection is secondary to the EL. C) The infection has pla ed an aetiological role in the development of EI None of these alternatives can be either proved or disproved by evidence available in single case. The last alternative awaits further confirmation from larger series, attempts at which ha e been made (14).

Although conclusive demonstration of a malignant viral oncogenesis in man has not as yet succeeded, acute leukaemia and Burkitt's lymphosarcoma are regarded as the nearest approaches to such a discovery *Mycoplasma orale* type I has been isolated from bone marrow specimens of patients with leukaemia by arious investigators, although none of these cases has been EL (1 11).

In the light of the circumstantial evidence of the disease reported here a *Mycoplasma* infection may be suggested as the cause, although the clinical course is radically different from that of infections known to be caused by *Mycoplasma*. This suggest a concomitant host response factor condition predisposing to this unusual course and/or another contributory aetiological agent. The absence of Hassall's corpuscles in the patient's thymus may imply a defective/altered immune state. Although the function of these corpuscles is still unknown, their absence has been noted in immunological deficiency disease such as the so-called Swiss type agammaglobulinaemia

Inasmuch as the absence of Hassall's corpuscles in leukaemia has not been investigated, the authors performed the following control

autopsied consecutive cases of acute leukaemia—one girl aged 5 years and four boys aged 11 months, 7 1/2 and 16 years—the thymus contained Hassall's corpuscles which were either calcified, cystic or normal. Other morphological changes in their thymus could be explained by administered antileukaemic therapy

Thus the present case differed from 5 other cases of acute leukaemia in regard to thymus morphology. The case of EL reported by Bengtsson (2) showed unexplained serological findings—a pronounced rise in the titres for antipneumolysin and Toxoplasma—and a tumour in the thymic region with metastases to lymph nodes and liver. With an impaired (immunological?) control against developing malignancy the response to mycoplasma or other antigens might lead to the development of leukaemia and not to "benign eosinophilia" (19).

To conclude, in the present case the criteria for EL given by Hauswaldt et al. (10) and Rickles and Miller (19) are fulfilled and other diagnostic alternatives are excluded. This seems to sustain the thesis of the existence of a separate disease entity called EL.

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DISACCHARIDASE ACTIVITY IN CHRONIC RENAL FAILURE

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Abstract. In 32 patients with chronic renal failure, average endogenous creatinine clearance 6.5 ml per minute (range 0–11), average age 45 years (range 19–58) and average duration of severe uremia 4.4 months (range 1–12) the following examinations have been carried out: lactose, sucrose and glucose-galactose tolerance tests, histological examination of jejunal biopsies and determination of the activity of lactase, sucrase, maltase and alkaline phosphatase in the mucosa. In 2 of the 32 patients there was evidence of lactase deficiency but this frequency is not higher than found in the general Danish population. Other tests were normal. It is concluded that affection of the small intestine is not an integral part of the chronic uremic syndrome.

Most patients with severe chronic renal failure have gastrointestinal symptoms. Almost any part of the mucosa in the gastrointestinal tract may be affected in uremia, but lesions are mainly localized to the stomach and the colon.

It is generally accepted that the small intestine is not affected by the uremic intoxication. This problem has only been sparsely investigated.

In this paper the function of the small intestine in uremic patients has been estimated by means of tolerance tests and measurements of the disaccharidase activities in jejunal biopsies, because these enzymatic functions are generally accepted to be damaged early in the course of small-intestinal involvement.

MATERIAL AND METHODS

Thirty-two patients with chronic renal failure with endogenous creatinine clearance less than 10 ml/min have been investigated.

Age and sex distributions are seen in Table I. The primary renal diseases are illustrated in Table II. The duration of uremia is defined as the period during which the serum creatinine has been permanently higher than 6 mg%. The average duration of uremia was 4.4 months (range 1–12).

Nineteen patients received modified Giovanetti diet, 9 were treated with intermittent peritoneal lavage and 4 were in chronic hemodialysis.

Gastrointestinal symptoms

Two patients suffered from typical peptic ulcer prior to renal disease. During the uremic state 3 patients developed typical ulcer dyspepsia with late postprandial pain relieved by ingestion of food and 3 others developed uremic nausea and anorexia. All patients had barium meal and in no patient was an ulcer found.

Diarrhea was present in only one patient, who suffered from uncharacteristic dyspepsia (patient 12). None of the patients investigated had observed food intolerance to milk products.

Tolerance tests

In all 32 patients, except one, tolerance tests with 50 g lactose, 25+25 g glucose-galactose and 50 g sucrose dissolved in 300 cm³ water were performed after an overnight fast. Blood sugar determinations by glucose oxidase method were carried out on capillary blood before and 15, 30, 45, 60, 90 and 120 min after administration of the sugars. Only one test was performed per day.

Inquiries were made about diarrhea and abdominal distress (colic, bloating, borborygmus) during and after the test.

Small-intestinal biopsies were obtained in 15 of the 32 patients. In all patients 3–4 samples were taken from the jejunum at or immediately distal to Treitz's ligament. Half of the biopsies were used for measurements of the activity of lactase, sucrase, maltase and alkaline phospha-

Table I. *Age and sex distribution*

	Age (y.)						
	<20	20–29	30–39	40–49	50–59	60	Total
Males	1	3	1	7	6	0	18
Females	0	0	2	7	5	0	14
Total	1	3	3	14	11	0	[illegible]

Table II *Renal diseases*

	Glomerulo-nephritis	Pyelo-nephritis	Polycystic kidneys	Other congenital malformations	M ximally contracted kidneys, diagnosis uncertain	Total
Males	8	1	3	3	3	18
Females	4	8	2	0	0	14
Total	12	9	5	3	3	32

tases and half for histological examination. The analyses were performed according to the method of Dahlqvist (2). The concentration f protein was determined by the method of Lowry (3) and the alkaline phosphatases as described by Bessey et al. (1). All enzyme activities are given in IU/g protein.

RESULTS

Table III shows the results of tolerance tests in 31 uremic patients and the enzyme activities in 15 small-intestinal biopsy specimens. In one patient (no. 6) only one biopsy was performed.

Lactose malabsorption was found in 2 patients (nos. 2 and 21). The blood sugar rise during the lactose tolerance test (ΔBS-LTT) was 18 and 11 g/100 ml respectively The glucose–galactose sucrose tolerance tests (GGTT and STT) were normal. In accordance with the tolerance sts the biopsy specimens also showed deficient mucosal lactase activity

The stereomicroscopic examination showed normal illi. With ordinary light microscopy hypercellularity mainly lymphocytes, in the lamina propria and hyperemia were seen in patient 2. Patient 21 revealed normal microscopy

During the tests neither of the 2 patients with lactose malabsorption had gastrointestinal symptoms. Two other patients (nos. 13 and 19) had diarrhea and borborygmus during LTT and one of them also during the STT despite the fact that their tests were normal The remainder of the patients were clinically unaffected by the tests.

The blood sugar rose rather late after the ingestion of sugar This phenomenon may be caused by delayed gastric emptying, a well known phenomenon in uremic patients.

Histology

Microscopically the 15 jejunal biopsies revealed normal mucosa in 7 patients. In all the biopsies normal fingerlike villi were found. The epithelium of the villi and the crypts appeared normal and the free surface was covered with a normal brush-border

The villus–crypt ratio and the number of mitoses were estimated to be normal.

Eight patients presented hypercellularity in the mucosa with increased number of lymphocytes and plasma cells. In the same patients a varying degree of edema in the top of the villi was found. Edema was not seen in the 7 patients in whom the mucosa was estimated to be normal. There was no significant correlation of these histological findings to the degree of uremia, the dietary regimen or dialytic treatment.

DISCUSSION

In 2 of 32 patients with severe chronic renal failure with endogeneous creatinine clearance below 10 ml/min evidence of lactose malabsorption was found. The diagnoses were confirmed by tolerance tests and determination of the enzyme activity in small-intestinal biopsies. The frequency 6–7% of lactose malabsorption does not differ significantly from the figures in the normal Danish population.

In 2 of 9 patients with chroni renal failure Riecken et al. (4) found villus atrophy and a general diffuse decrease of the enzyme activity in the resorptive epithelium. The average villus height was found to be significantly reduced and the mucosa thickness was significantly increased. Moreover there was a rise in mitotic counts, reflecting a hyperregenerative state analogous to celiac disease This mucosal change improved after treatment with intermittent hemodialysis in the course of a few months The degree of renal insufficiency is not mentioned in the work of Riecken et al.

Table III. *Tolerance tests in 31 uremic patients and disaccharidase activities (IU/g protein) in small-intestinal biopsy specimens from 15 patients*

∆ BS = blood sugar rise (mg/100 ml). LTT = lactose tolerance test, STT = sucrose tolerance test, GGTT = glucose-galactose tolerance test

Pat. no.	Sex	Age (y.)	Duration of uremia (mo.)	∆ BS–LTT	∆ BS–GGTT	∆ BS–STT	Lactase (9–98)	Sucrase (26–130)	Maltase (111–420)	Alkaline phosphatases (IU/g protein)
1	♂	42	1	65	100	46	18	40	135	422
2*	♂	48	5	18	44	59	1.6	21	73	495
3	♂	45	2	66	56	55	14	34	116	589
4	♂	29	3	81	132	89	16	31	104	388
5	♂	45	3	56	69	61	18	33	110	540
6	♂	49	1	—	—	—	75	142	408	905
7	♀	35	4	59	65	80	19	67	251	515
8	♀	47	3	65	68	79	31	77	255	743
9	♂	51	1	60	66	93	20	37	123	461
10	♀	49	1	118	160	140	27	63	247	561
11	♀	37	1	32	55	70	27	60	203	1 39
12	♂	33	12	73	35	60	32	70	200	87
13	♀	37	1	43	76	114	—	—	—	—
14	♀	48	3	87	96	128	—		—	—
15	♂	50	3	30	37	53	—	—	—	—
16		42	12	46	45	77	—	—	—	—
17	♂	51	3	52	78	73	—	—	—	—
18	♀	53	6	52	56	53	—	—	—	—
19	♀	43	7	81	96	140	—	—	—	—
20	♀	38	3	56	73	81	—	—	—	—
21	♂	30	12	11	91	95	0.74	14	58	49
22	♀	32	3	72	95	111	40	79	310	1 128
23	♂	19	12	65	110	85	—	—	—	—
24	♂	42	8	78	56	117	21	43	175	995
25	♂	27	6	65	49	40	—	—	—	—
26	♀	48	3	63	58	62	—	—	—	—
27	♂	38	3	65	74	65	—	—	—	—
28	♂	49	3	56	59	60	—	—	—	—
29	♀	58	3	78	78	58	—	—	—	—
30	♂	54	6	83	70	106	—	—	—	—
31	♂	31	6	56	49	70	—	—	—	—
32	♀	43	2	81	76	63	—	—	—	—

* Lactose malabsorption.

Rocha et al. (5) investigated 14 patients with chronic renal failure with average endogenous creatinine clearance of 9.4 ml/min. The examination included jejunal biopsies and in a few of the biopsies they found increased numbers of lymphocytes and plasma cells in the lamina propria. The height and configuration of the villi were normal.

We did not find the mucosal and histochemical changes as indicated by Riecken et al. The small-intestinal biopsies in all our cases were taken at or immediately distal to Treitz's ligament. In three of the cases we ha e also biopsies from the second part of the duodenum. In one of these the villi were found to be atrophic with reduced height and in the two others they were flattened and varied much in shape. We consider this normal physiological variation.

As mentioned above, two cases in the present series had severe lactase deficiency and a flat blood sugar curve following oral lactose ingestion. The same two patients also had subnormal mucosal sucrose and maltase levels. However their sucrose tolerance test showed a normal rise of blood glucose which precluded the presence of sucrose malabsorption.

Rubin and Dobbins (6) state that the morpho-

logy of the small-intestinal mucosa varies with the location from which the biopsy is obtained, and it is stressed that the duodenal villi may be broader and more branched than jejunal villi and may even be absent.

In all our three cases with duodenal biopsy abnormalities the villi from Treitz's ligament were normal. In 8 cases edema of the villi was found and there was simultaneously increased cellularity in the lamina propria. This hypercellularity is generally considered to be a non-specific reaction.

No correlation was found between the hyper cellularity and the degree of renal insufficiency, Hb level, renal disease or duration of uremia.

It is concluded that in the 32 uremic patients investigated no evidence of enzymatic or significant morphological abnormalities was present.

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GLOMERULAR FILTRATION RATE, RENAL PLASMA FLOW AND FILTRATION FRACTION IN LIVING DONORS BEFORE AND AFTER NEPHRECTOMY

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Abstract. Simultaneous inulin, ^{125}I-iothalamate, creatinine, PAH and ^{131}I-hippuran clearances have been performed in 18 prospective living kidney donors. Good correlation was found between inulin and ^{125}I-iothalamate and between PAH and ^{131}I-hippuran clearances as well as between the above mentioned clearances and renal size. In 13 instances ^{125}I-iothalamate, creatinine and ^{131}I-hippuran clearances were carried out before and 26–53 months after unilateral nephrectomy. An increase was found in ^{125}I-iothalamate, creatinine and ^{131}I-hippuran clearances of 66, 67 and 31 respectively. These increases were independent of the age (range 19–65 years) and sex of the donor. Clearance values were however well correlated to the size of the remaining kidney. Pattern of kidney function changes, the filtration fraction being significantly greater postoperatively.

The purpose of the present methodological study of renal function was to investigate changes in the remaining kidney's function after unilateral nephrectomy in individuals with normal renal function. Living renal donors were studied and the glomerular filtration rate (GFR), renal plasma flow (ERPF) and filtration fraction (FF) were determined before and after nephrectomy.

Since the 1930's (6, 25, 26, 31) inulin and para-aminohippuric acid (PAH) have been used to determine GFR and ERFP. During recent years these traditional reference substances have been replaced to an increasing degree by radioactive tracer materials. ^{125}I-iothalamate clearance has been shown to be identical with inulin clearance (7, 17, 27, 28). In 1945 Smith et al. (30) demonstrated that hippuran clearance was identical with PAH clearance. In 1960 ^{131}I-hippuran was produced (36, 38) and later studies have shown a good correlation between PAH and ^{131}I-hippuran clearances.

In dogs rapid hypertrophy and hyperplasia have been shown to take place in the remaining kidney after unilateral nephrectomy (3) and GFR and ERPF increase to about 80% of the preoperative value in the course of a month. Also in man the GFR and ERPF began to increase within 7 days after unilateral nephrectomy (8, 15, 4).

Kidney function was studied by means of the traditional inulin and PAH clearance technique. Prior to nephrectomy simultaneous creatinine, inulin, ^{125}I-iothalamate, PAH and ^{131}I-hippuran clearances were carried out. Postoperatively creatinine, ^{125}I-iothalamate and ^{131}I-hippuran clearances were performed simultaneously.

MATERIAL

Prior to nephrectomy 18 donors, 10 women and 8 men, were studied. BP, Hb and serum electrophoresis were normal in all. None had evidence of cardiac or pulmonary disease. None had proteinuria or glucosuria. Fasting blood sugars were normal. Renal concentrating ability was normal as judged by determination of urine osmolality during 26-hour concentration test. Other data are given in Table I. In 13 donors clearance studies were carried out before and after nephrectomy. Nephrectomy was performed without any complications. Data are given in Tables II and III.

METHODS

Inulin (Inutest, Laevosan). Priming and sustaining doses were given to achieve plasma concentration of inulin between 15 and 25 mg/100 ml. Inulin was measured by Heyrovsky's method (14).

PAH (Sodium Aminohippurate, MSD) was used to determine ERPF. The plasma concentration of PAH was between 1.5 and 2.0 mg/100 ml during the study period. PAH was measured by Bratton and Marshall's method (1).

^{125}I-iothalamate and ^{131}I-hippuran (Amersham Radio-

Table I *Kidney function studies in donors before nephrectomy*

$C_{In} = C_{inulin}$, $C_{Io} = C_{131I\text{-}iothalamate}$, $C_{131I\text{-}h} = C_{131I\text{-}hippuran}$
All clearances are corrected to standard body surface (ml/min/1 73 m²)

Donor no.	Age (y)	Sex	C_{In}	C_{Io}	C_{Cr}	C_{In}/C_{Io}	C_{Cr}/C_{Io}	C_{PAH}	$C_{131I\text{-}h}$	$C_{PAH}/C_{131I\text{-}h}$	C_{In}/C_{PAH} 100	$C_{In}/C_{131I\text{-}h}$ 100
74	46	♀	85	89	102	0.96	1 15	457	437	1.05	18.8	20 9
84	47	♂	105	119	105	0.96	0 88	407	401	1.00	25.8	27.2
93	60	♀	100	101	93	0.99	0 92		384			26 3
100	25	♀	83	100	106	0 83	1.06	586	501	1 17	14 2	20 8
145	60	♀	82	81	123	1.01	1.51		318			25.5
48	4	♂	91	88	99	1.04	1 13	301	349	0 85	30.2	26.2
77	49	♂	74	83	107	0.97	1 35	342	343	1.00	21.6	4 2
78	50	♂	119	113	135	1.06	1 19	518	402	1.28	23 0	28 8
79	42	♂	101	82	134	1.26	1.61	715	650	1 10	14 1	12 6
81	44	♂	98	101	88	0.97	0.91	667	633	1.07	14.7	15 9
86	60	♂	115	128	118	0.91	0.93	572	492	1 19	20.1	26 8
88	52	♀	87	87	87	1.00	1.00	371	352	1.05	23.4	24.7
89	33	♀	60	71	87	0 85	1.23		309			23 0
91	19	♀	91	104	102	0.90	0 96		489			21.3
94	44	♀	72	79	112	0.91	1.42	462	461	1.00	15.6	17 1
95	40	♀	89	102	135	0.88	1.30		523			19 5
109	65	♂	66	60	103	1 10	1.59		301			19 9
111	56	♀	93	100	103	0.93	0.96	630	533	1 18	14.8	18 8
Average	46		89.5	93 8	107 7	0 97	1 17	502	438	1.08	19 7	[illegible]
S D			15.7	17.0	15.7	0.10	0.24	133 8	105 4	0.12	5.3	4 3
S.E.M			3.7	4 0	3.7	0.02	0 06	38 6	24.9	0.03	1 5	1 0
Range			60–119	60–128	87–135	0 83–1.26	0.88–1 61	301 715	301 650	0 85–1.28	14.1 30 2	12 6–28 8
Coefficient of variation			12.08	9.58	10.29			10.15	8.85			

Table II *Donor data*

Donor no.	Age (y)	Sex	Months post nephrectomy	Remaining kidney	Kidney size prenephrectomy: Total area of both kidney	Remaining area
48	28	♂	53	Left	191.6	96 6
77	52	♂	40	Left	157 4	83 6
78	53	♂	40	Right	—	—
79	45	♂	40	Right	192.5	91 0
81	47	♂	39	Left	186 9	94 5
86	63	♂	37	Left	204 0	104 0
88	55	♀	35	Right	144.8	72.5
89	56		35	Right	118.7	52.6
91	22	♀	35	Right	114 8	53.8
94	47	♀	34	Right	133	63 0
95	43	♀	32	Left	149 4	67.8
109	67	♂	28	Right	168.8	67.5
111	58	♀	26	Right	179 9	99.–
Average	47		36		157 7	75.9
S.D					30.82	17.99
No./S.E.M		♀ 6 ♂ 7		Left 5 Right 8	8 90	5 19

Table III. *Kidney function studies in donors before and after nephrectomy*

Corr clearance ml/min/1 73 m² Not corr = clearance ml/min not corrected t st ndard body surfac

	$C_{iothalamate}$				$C_{creatinine}$				$C_{131I\ hippuran}$				C_{Cr}/C_{iotha}		C_{In}/C ×100	
	Before		After		Before		After		Before		After					
onor	Corr.	Not corr	Corr	Not Corr	Corr	Not corr	Corr	Not corr	Corr	Not corr	Corr	Not corr	Before	After	Before	After
48	88	98	72	80	99	110	96	107	349	386	296	328	1 13	1 33	26 2	21 3
77	83	81	67	70	107	112	58	62	343	336	212	255	1 35	0 86	24	31
78	113	130	78	95	135	155	91	111	402	462	232	282	1 19	1 16	28 0	31
79	82	82	97	100	134	134	95	98	650	650	342	352	1 61	0 98	12 6	28 4
81	101	106	89	98	88	93	94	102	633	666	413	448	0 91	1 06	15 9	21 5
86	128	136	96	100	118	125	98	102	492	523	358	372	0 93	1 03	6 0	26 8
88	87	84	69	65	87	84	79	75	352	338	212	200	1 00	1 14	24 7	32 5
89	71	62	71	62	87	76	76	66	309	271	196	170	1 20	1 07	23 0	36 2
91	104	93	94	84	102	91	95	85	489	436	318	283	0 96	1 01	21 3	2 [illegible]
94	79	71	52	46	112	100	83	73	461	412	229	202	1 42	1 40	17 1	22
95	102	89	87	77	135	118	89	77	523	459	310	269	1 30	1 02	19 5	28 1
	60	70	58	67	103	120	78	90	301	350	276	317	1 59	1 34	19 9	21 0
	100	76	85	64	103	79	106	79	533	403	283	212	0 96	1 25	1	[illegible]
Average	92.2	90 6	78 1	77.5	108.5	107.5	87.5	86.7	449 0	437.8	282 0	283 8	1.20	1 14	21	25
S D	18 21	22 29	14.59	17 14	17.55	23.19	12.51	16.15	117.2	117.5	65 41	79.23	0 24	0 20	4 5	4
S.E.M	5 05	6 18	4 05	4.75	4 87	6 43	3 47	4 48	32.5	32 6	18.14	21 97	0 07	0 05	1 2	1

cal Pharmaceuticals) are stored in the dark at for maximum of 14 days. The amount of free odine in the specimens analysed was less than 2%. Sufficient ¹²⁵I-iothalamate was given to assure plasma con between 800 and 1 800 p m./ml. The concentration of ¹³¹I-hippuran in the plasma was between and 600 p.m./ml. The activity of the radioactive icers was measured in γ-counter provided with pulse height analyser to statistical inaccuracy of ess than 1%.

Creatinine was determined with AutoAnalyzer (37).

The classical clearance technique with constant infusion given by Goldring and Chasis (9) was used. Clearance studies were performed in the morning in fasting subjects. In order to increase urine output to around 10 ml/min or 300 ml/hour was given beginning 2 hours after the start of the study. Blood and urine specimens for nation of blind values were obtained hour re the first clearance period. All patients wer subjected to 3 clearance periods lasting approximate Blood specimens were removed 5 min before the of each clearance period (12). Urination took place the standing position at the bedside. On the basis I renal angiography performed before transplantation determination of the kidney was calculated as given Moll (22). Spearman' test was used to compare al function and renal size before and after nephrec-

RESULTS

from the 19 donors before nephrectomy are in Table I. All result are the average of 3 consecutive clearance studies. In lo reve d an a erage of 0.97 ± 0 10. Regression analy demonstrated no differences in filtration mea ured with the two methods (Fig. 1). Cr/I w 1 17 ± 0.24. The regression analysis is show in Fig. 2. There was a statistically significant difference between creatinine clearance and ¹²⁵Iothalamate clearance (0 02 > p > 0.01). ¹²⁵I/PAH I hippuran was 1 09 ± 0.12. The regression analysis is shown in Fig. 3. The FF In/PAH v ried greatly with average values of 1/7 ± 3 Ir/¹³¹I-hippuran was 22 1 ± 4.3

On investigation 26–53 months after nephrectomy (Tables II and III) ¹²⁵Iothalamate clearances averaged 78 1 ml/min ± 14 6 in contrast to 92.2 ml/min ± 18 2 before nephrectomy. There was a positive, statistically significant correlation between values before and after nephrectomy (0.01 > p > 0.001). Creatinine clearance before nephrectomy was 108 ml/min ± 17 6. After nephrectomy average creatinine clearances were 87.5 ml/min ± 12.5. There was a posit ve non-significant correlation between va ues before and after nephrectomy.

Before nephrectomy ¹³¹I-hippuran clearance was 449 ml min ± 117 after nephrectomy 282 ml min ± 65 4. There was a posit ve statistically

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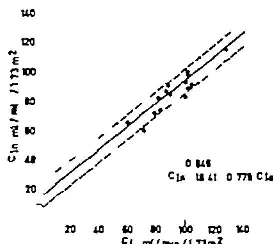


Fig 1 Inulin clearance compared to [125]Iothalamate clearance, showing the fitted regression line (—) and the S.D ( ).

significant correlation between values before and after nephrectomy ($0.001 < p < 0.005$).

On the basis of area calculations, 48% functioning renal tissue remained after nephrectomy [125]Iothalamate, creatinine and [131]I-hippuran clearances were correlated to the size of the remaining kidney ($0.005 < p < 0.01$). Assuming that ... of the functioning renal mass had been re...ed [125]Iothalamate clearance increased by ...% ± 29 creatinine clearance by 64% ± 30 and [131]I-hippuran clearance by 32% ± 24 Thus there was a significant increase in the function of the remaining kidney The increase in renal function was independent of age, sex and time of study after nephrectomy The FF increased from 21.3 ± 4.5 preoperatively to 28.2 ± 4.8 after nephrectomy This change was statistically significant ($0.01 > p > 0.005$).

DISCUSSION

Reported values for filtration as measured by inulin clearance have varied from 66.5 to 199.5 ml/min (2, 4 23 32). Inulin clearance in our study was found to be within the lower normal range. Eleven (61%) of 18 donors were older than 45 years of age 13 had an inulin clearance < 100 ml/min, but it is well known that filtration decreases with age (4). The results of simultaneous inulin and [125]Iothalamate clearances showed good correlation, as has been previously demonstrated (7 23 27 28).

It is usually assumed that creatinine clearance represents GFR. As a rule $^{C}Cr/In$ is found to be approximately 1 0 but variations of 0.73–1.50 are seen in these clearance ratios (21 33 34). In the present study $^{C}Cr/Io$ was between 0 88 and 1 61 with an average of 1 17 The reason for varying ratios in healthy individuals is not completely clear but part of the explanation lies in the methods used for analysis. Healy (13) has shown that, regardless of whether creatinine total chromogen or creatinine measured with AutoAnalyzer is used, filtration is overestimated by creatinine clearance The factors which influence filtration (hydration, BP Hct, colloid-osmotic pressure and temperature) were normal in all individuals studied thus the values for GFR measured by

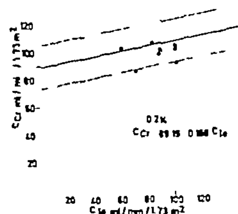


Fig 2 Endogenous creatinine clearance compared to [125]Iothalamate clearance. Symbols as in Fig. 1.

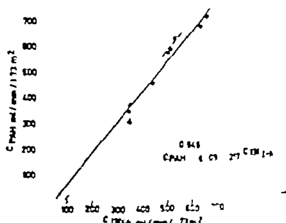


Fig. 3 PAH clearance compared to [131]I-Hippuran clearance. Symbols as in Fig. 1

inulin, [125]I-iothalamate and creatinine clearances represent the normal range in our laboratory

Values for ERPF also vary a great deal. Diodrast clearances range from 282 to 1 105 ml/min (2, 4 32). At the age of 50 average values are around 530 ml/min. In 29 donors with an average age of 36 years Krohn et al. (15) found a PAH clearance of 521 ml/min (range 352–782). Six of them (20 7 %) were older than 45 years and had an average PAH clearance of 428 ml/min. In 17 donors with an average age of 39.3 years Ogden (24) found a mean PAH clearance of 476 ml/min. Four of them were older than 45 years and had an average PAH clearance of 463 ml/min.

In our study 7 of 12 donors (58.3 %) were older than 45 years and had an average PAH clearance of 471 ml/min. The other 5 had a PAH clearance averaging 546 ml/min. PAH clearance in our study was thus in agreement with the findings of others. Eleven of the 18 prospective donors were older than 45 years and had an average [131]I-hippuran clearance of 388 ml/min. The remaining 7 had an average [131]I-hippuran clearance of 515 ml/min.

According to Smith et al. (30) hippuran clearance is identical with PAH clearance. It is, however well known that hippuran clearance measured with [131]I-hippuran given in tracer dose amounts is approximately 10 % less than PAH clearance (36). The reason for this is unknown. It has been suggested that [13] I-hippuran is more strongly bound to protein than PAH (16, 17 20 35). Factors concerning protein binding have not yet been completely elucidated (19). The amount of free iodine will influence clearance since iodine is approximately 25 % of creatinine clearance (29). We found free iodine to be <2 % In the present study there was good correlation between PAH and [13] I-hippuran clearances.

The size of the FF is also reported at varying levels. Bran et al. (2) ga e ranges of 11 0–31 4 and Smith et al. (32) of 10.9–29.5 Values for In/PAH and [125]Io/[13] I-hippuran were thus within the normal limits in the present study

In animal studies a rapid increase in GFR and ERPF has been demonstrated after unilateral nephrectomy This change in function takes place from a few hours to a few days after nephrectomy (10, 18). After unilateral nephrectomy the kidney of the mouse increases in size and rat glomeruli become hyperemic within a minute. The primary reason for this change is an increased blood flow to a reduced population of nephrons, but in the course of a few hours to a few days hypertrophy and hyperplasia take place in the remaining renal tissue. The renal mass of the rat increases 17 % during the first 4 hours and in the course of a week the kidney size has increased 30–40 % The increase in kidney size in the dog is less marked. Rises in GFR and ERPF in dogs, which take place in connection with unilateral nephrectomy and functional elimination of one kidney are partly independent of increases in the functioning renal mass (3).

Krohn et al. (15) studied 29 donors within the first 18 days after nephrectomy They found that the endogenous creatinine clearance and the PAH clearance were 71 and 70 % respectively of the preoperative values. Flanigan et al. (8) found that the GFR in 24 donors 6 months after nephrectomy was 61 ± 5 % of the value before nephrectomy and that the ERPF was 57 ± 7 % of the preoperative value. A study of 4 donors 1 –19 months after nephrectomy by Donadio et al (5) showed that diatrizoate clearance was 72–97 % and hippuran clearance 61–77 % of the preoperative values. Ogden (24) found that creatinine clearance was 70 % of the preoperative value. These studies were undertaken 21–48 months after unilateral nephrectomy The findings of the above mentioned investigators are thus in agreement with findings from animal experiments.

We studied 13 donors 26–53 months after nephrectomy Considering that the functional renal mass was reduced by 50 % we found that the remaining kidney function was significantly increased with regard to both GFR and ERPF The percentage increase of GFR was twice as great as the increase in ERPF This increase in function was independent of age and sex and was not related to the time of study within the 26–53 months after nephrectomy There was a positive, statistically significant correlation between clearance values after nephrectomy and the size of the remaining kidney measured before nephrectomy It can be taken as evidence that the increase in function was relatively uniform and dependent to greater degree on the renal mass remaining after nephrectomy than on age, sex and time of study This is in contrast to the findings of Ogden (24) who reported an inverse

correlation between age and ability to increase renal function after unilateral nephrectomy He found in donors over 45 years of age that creatinine and PAH clearances increased 13 and 10% respectively after nephrectomy In our studies [125]Iothalamate, creatinine and [131]I-hippuran clearances increased 66 67 and 31% in this age group

The functional profile was also altered in that the FF was significantly higher postoperatively Because BP degree of hydration, circulatory status and weight were not significantly different between the two study periods, these factors cannot have influenced the results. The higher FF after unilateral nephrectomy suggests a change in the hemodynamic pattern, affecting afferent and efferent arterioles provided that membrane characteristics are unchanged. This is supported by renographic studies in unilaterally nephrectomized donors in whom a change in the parameters measuring the relationship between speed of uptake and release was very slight (12). Changes in FF were independent of age sex and time of study

Thus it can be concluded that, if the above mentioned parameters of renal function are normal, the chronological age and sex of the donor of no importance for kidney function after nephrectomy

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DC ELECTROCONVERSION OF PATIENTS WITH ATRIAL FIBRILLATION ADMITTED TO A CORONARY CARE UNIT

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Abstract. A retrospective study of patients admitted to coronary care unit (CCU) because of suspected acute myocardial infarction (AMI) and subjected to DC electroconversion for atrial fibrillation (AF) is presented. DC electroconversion was resorted to in 35 patients. In 19 diagnosis of an AMI was made. All but two of the 16 remaining patients had findings or symptoms of ischaemic heart disease. The two patient groups, i.e. those with and without diagnosis of AMI, behaved similarly as regards response to DC electroconversion. Twenty-nine of the 35 patients (83%) developed sinus rhythm (SR) at electroconversion. However AF again developed in the unit in 11 of these 29 patients and occurred in 7 within few minutes of DC electroconversion. Without further attempts at DC electroconversion 80% of the 35 patients were in SR at time of discharge from the CCU. The most striking benefits were observed in patients in whom hypotension was the major reason for resorting to DC electroconversion. The only complication observed was one instance of ventricular fibrillation, apparently caused by the DC shock, which responded to defibrillation.

The improved management of the arrhythmias complicating acute myocardial infarction (AMI) has probably been a major cause in reducing the early hospital mortality in this condition. However, comparatively little attention has been paid to the management of atrial fibrillation (AF) complicating AMI which, according to some authors, is associated with raised mortality (1, 7, 15). Furthermore, only occasional reports, including but few patients, have appeared on the effects of DC electroconversion in this situation (4, 5, 7).

For these reasons a retrospective study has been performed with the purpose of describing the results obtained when treating these patients with DC electroconversion. Furthermore as the diagnosis of AMI is commonly not proven when performing DC electroconversion in a CCU the results are included also from patients who subsequently were found not to have AMI.

METHODS

Serafimerlasarettet, which is teaching hospital, served an undefined population within greater Stockholm in 1968–70. A more defined population from certain districts has been served since Jan. 1, 1971. Further details regarding the policy of the CCU of this hospital, its admission and diagnostic criteria were given in previous publication (10).

Treatment of atrial fibrillation. The treatment of AF was primarily guided by the ventricular rates. Patients with rapid ventricular rates (≥120/min) were treated with digitalis (deacetyl-lanatoside-C, 0.4–0.8 mg, or ouabain, 0.25–0.375 mg i.v.) which, if not successful in reducing the ventricular rates within 2 hours, was supplemented by DC electroconversion (100–300 J) under light general anaesthesia. Frusemide, 20 mg or more i.v. was often given. In the presence of severe haemodynamic dysfunction, e.g. cerebral symptoms, hypotension, heart failure or anginal pains DC electroconversion with the electrode paddles in the anteroposterior position was immediately performed under general anaesthesia or diazepam sedation. Chronic AF or AF of recurrent type were not treated with DC electroconversion.

MATERIAL

During the period of study i.e. Jan. 1, 1968, until June 6, 1971, 2 028 patients were admitted to the CCU because of symptoms suggesting AMI. DC electroconversion for AF was resorted to in 35 patients. In 19 of these diagnosis of AMI was made, whereas the other 16 failed to develop either characteristic enzyme or ECG changes (Fig. 1). The latter will be referred to in the sequel as non-AMI patients. Altogether 315 (15%) of the AMI and non-AMI patients had AF during their stay in the CCU showing that, with the criteria given

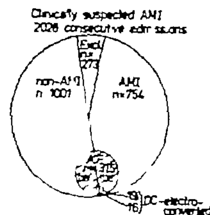


Fig 1 Diagram showing different subdivisions of the patient material and how the group subjected to DC electroconversion as obtained. Excl. = excluded as they fulfilled neither AMI nor non-AMI criteria.

for selection of patients for DC electroconversion, this treatment became pplicable in 11% of patients ith AF Patients with atrial flutter has not been included in this study

The age and sex characteristics of the 35 patients investigated ar given in Table I. The indications for resorting to electrocon ersion are shown in T ble III.

RESULTS

Delay The mean delay between onset of symp- and admission was 6 hours for the AMI and 5.5 hours for the non-AMI group. AMI group 13 patients (68%) had been -ted to DC electroconversion within 6 hours onset of symptoms of AMI, and 17 (89%) within 1 hours. The corresponding figures for the non-AMI group was 9 patients (56%) within 6 hours and 11 (69%) within 1 hours.

Mortality Six (3?%) of the DC electroconverted AMI patients died during hospitalization, 3 in the CCU The mortality of the non-AMI patients was 3 (19%), one patient having died in the CCU and the two others during after-care. The cause of death for the non-AMI patients was progressive heart failure in two and pulmonary embolism in one. Mortality was not related to response to DC electroconversion.

Past history The findings are summarized in Table I. It is noteworthy that all but two of the non-AMI patients had a history of ischaemic heart disease All but three of the patients with a history of previous heart failure defined as having had treatment for heart failure were on digitalis at the time of admission. One of the AMI and four of the non-AMI patients had a systolic murmur suggesting mitral regurgitation, probably of non-recent onset in all. None of these factors were related to the results of attempted DC electroconversion.

ECG and enzymes In the AMI group 9 patients

Table II. *ECG findings and SGOT maximum levels in 19 patients with AMI complicated by AF subjected to DC electroconversion*

	No. of pats.
ECG	
Non-conclusive	6
Anterior	5
Lateral	1
Inferior	2
Anterolateral	2
Inferolateral	2
SGOT maximum	
40-100	7
101 200	5
201 300	4
> 300	2
Not obtained	1

Table I. *Age sex and past cardiac history of 35 patient admitted because of suspected AMI and treated ith DC electroconversion because of AF*

	All pats. (35)	AMI pat (19)	Non-AMI pat (16)
Men	28	16	1
Women	7	3	4
Age range (y)	50-84	58-84	50-76
Angina pectoris	20	10	10
Myocardial infarction	18	8	10
Heart failure	23	11	12

Table III. *Indications for DC electroconversion in 35 patient with AF admitted because of suspected AMI*

	AMI pat (19)	Non-AMI pat. (16)
Hypotension	7	3
Hypotension congestive heart failure	3	
Congestive heart failure	2	4
Chest pain	1	3
Non-response to medical therapy for high ventricular rates (120 min)	6	6

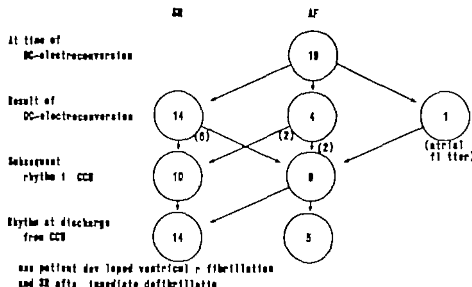


Fig. 2. Results of attempted DC electroconversion of AF complicating the early phase of an AMI in 19 patients. The subsequent course in the CCU is also shown.

(47%) had ECG findings which did not fulfil our criteria for an acute infarction. In these patients the diagnosis was supported by characteristic enzyme elevations. The findings are summarized in Table II. In the non AMI group 3 patients had left bundle branch block, 4 had normal ECG findings apart from the arrhythmia, and in the remaining patients various abnormal findings were seen.

By definition no characteristic rise of enzymes suggestive of an AMI was seen in the non-AMI patients. The findings in the AMI group are given in Table II. No representative blood samples were available in one patient, as he died prior to calculation of the maximum enzyme rise. No relationship between infarction site and enzyme rise with results of DC electroconversion was found.

Indication for DC electroconversion. In both AMI and non-AMI groups the most common reason for resorting to DC electroconversion was non-response to medical treatment in patients with AF and high ventricular rates. The symptoms and findings leading to DC electroconversion are presented in Table III. No relationship between success rates of attempted electroconversion and the indication for this treatment was found.

Maximum energy levels. The maximum energy levels used in both groups have been related to the results of the treatment. Generally energy levels have been increased by 100 J increments to 300 J. In 13 patients 100 J sufficed to give SR. In two patients energy levels were increased to 400 J and SR was obtained in both. No recognizable pattern could be discerned as regards rate of reversion to SR or its permanency when related to the maximum energy levels required.

Therapy prior to DC electroconversion and reversion rates to sinus rhythm. SR was obtained at DC electroconversion in 14 (74%) of the AMI patients. In one further patient atrial flutter developed. This is schematically presented in Fig. 2. SR after DC electroconversion lasted only for a few minutes in four patients, about one hour in one patient and 12 hours in another patient prior to relapse to AF. One patient developed ventricular fibrillation at DC electroconversion, possibly due to defective triggering. Defibrillation resulted in SR. In the AMI group 15 patients (79%) had received digitalis and frusemide, two only digitalis, one nly frusemide and one neither prior to attempted DC electroconversion. In three cases slow-release quinidine was given. Several patients were also on i.v. lidocaine.

DC electroconversion resulted in SR in 15 (94%) of the non-AMI patients. One patie

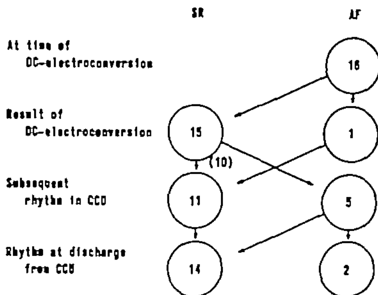


Fig. 3. Results of attempted DC electroconversion of AF in the 16 non-AMI patients. The subsequent course in the CCU is also shown.

veloped SR without further attempt at DC electroconversion subsequently (Fig. 3). In 5 patients the SR obtained could not be maintained. Three patients developed AF within a few minutes of DC electroconversion, one after 12 hours and the last after 3 days. Digitalis and frusemide were given to 7 patients (44%) in the non-AMI group prior to attempted DC electroconversion. Digitalis only was given to 3 patients and frusemide only 4. Two patients received procaine amide, one idine and another verapamil. Several patients on lidocaine therapy

of DC electroconversion on patients with hypotension. Seven of the AMI patients (Table III) and 3 of the non AMI patients were hypotensive (≤90 mmHg systolic) and this was either the only or the major indication for DC electroconversion.

Five of the AMI patients responded by a higher systolic BP (≥100 mmHg) after converting to SR. One of these patients was at this time being treated with intraaortic counterpulsation and the pressure rise could be confirmed by direct intra-arterial measurements (9). In two patients in whom DC electroconversion could not affect AF no haemodynamic improvement was seen. In three further instances with AMI, both hypotension and congestive heart failure were considered to be indications for DC electroconversion one patient having frank pulmonary oedema. They were all in a very poor condition and subsequently died. SR was obtained in only one.

All three non-AMI patients with hypotension responded to DC electroconversion with SR and, in all, systolic pressures rose to ≥100 mmHg.

Further course. This is also seen in Figs. 2 and 3. Considering the AMI patients first, SR was maintained throughout the CCU stay after DC electroconversion in 8 patients (42%), one of whom died in the unit. In the remainder of the AMI group varying patterns of return to AF subsequent reversal to SR, etc. were seen (Fig 2). After DC electroconversion 10 patients were given digitalis and slow release quinidine. At the time of discharge from or death in the CCU 14 patients were in SR and 5 in AF. One patient was in SR and two in AF prior to death. At the time of discharge from the CCU the 16 alive patients showed AF in 3 instances and SR in 13.

In the non-AMI group (Fig. 3) a rather similar pattern was seen and no significant differences were found when comparing these patients with the AMI group. In all but one of the 16 patients SR was established by DC electroconversion and in 10 was maintained until discharge from the unit. The patient who did not respond to electroconversion developed SR in the CCU subsequently. Five of the 15 patients who had responded to DC electroconversion reverted to AF in the unit but three then again developed SR prior to discharge. Fifteen of the 16 patients were discharged alive from the CCU and 14 were in SR.

Adding the two groups together ($n = 35$) the following findings emerge. DC electroconversion irrespective of final diagnosis resulted in SR in 29 patients (83%). Against this must be set the fact that the SR obtained is relatively unstable and in 38% (11/29) AF again developed during the stay in the unit occurring within a few minutes in 7 patients. In several of these patients, however SR again developed without need for further attempt at DC electroconversion. Altogether 80% of the patients were in SR prior to leaving the CCU Apart from the patient who developed ventricular fibrillation no certain adverse reactions could be shown to have been caused by DC electroconversion.

DISCUSSION

DC electroconversion is probably the method of choice for converting AF to SR. However in patients with AF complicating an AMI hesitancy has been expressed as to the usefulness of this procedure (5 7). This is partly due to the transitory nature of this arrhythmia in AMI and probably also to the fact that several reports have failed to demonstrate a worsened prognosis in patients with AF in AMI (3 5 6, 16). Hesitancy has also been felt owing to the possibility of DC electroconversion as a potential cause of myocardial damage, and this has received some support in enzyme studies after DC electroconversion although doubts as to the origin of the enzymes remain (12, 14). Additional fears have been nourished by reports of heart failure after DC electroconversion (2, 13). However reports have now appeared of a raised mortality in patients with AMI complicated by AF (7 9 15) and this was also found to be the case in our CCU (4). To what degree AF per se is responsible for this worsened prognosis remains doubtful owing to the close relationship of this arrhythmia to the incidence of heart failure and age (4).

The present retrospective study cannot provide a simple answer concerning the relative merits of DC electroconversion as opposed to other forms of management of AF in this situation. For such purposes a controlled study would be required. Some observations of clinical value have, however emerged. Patients admitted according to the usual criteria employed in many CCUs, but who neither develop a characteristic rise of enzymes nor ECG signs suggestive of an AMI (i.e. the non-AMI patients), behave in a rather similar manner to those with a subsequently verified AMI. It would therefore seem that it is not an underlying AMI per se which should cause the physician to hesitate as regards DC electroconversion. On the other hand this study has failed to identify which patients with AF will respond to DC electroconversion with sustained SR in this situation.

The rate of success as regards DC electroconversion as measured by the number of patients developing SR was in fact higher than would have been anticipated from the reports that may be found in the literature (4 5 7) as 83% of the patients developed SR. This can be compared to the corresponding results of 84 consecutive attempts to electroconvert chronic AF in this hospital in 1969–70. Of these patients 84% developed SR. This latter figure is in accordance with other reports (8).

The figure of 83% in the present series may appear misleading. As was shown above, 7 patients had SR for only some minutes after attempted DC electroconversion and 4 of them died subsequently If these 7 patients are also considered as failures, and therefore subtracted, SR of some duration was in fact only obtained in 63% In the group with chronic AF referred to above, similar short-lived SR was registered in 3 patients.

Another consideration as regards the usefulness of DC electroconversion in these patients must be those in severe haemodynamic distress as shown by shock, hypotension or severe heart failure. Even a short-lived success providing added atrial transport may improve the situation in these patients. These aspects, unless evaluated with more sophisticated haemodynamic measurements, are hard to estimate quantitatively Even so, it would appear that patients with hypotension as the major indication for DC electroconversion of the arrhythmia gained considerable benefit when SR was obtained. Although it is impossible to know how these patients would have fared without DC electroconversion, it is tempting to suggest that this may be a very strong indication for DC electroconversion in patients with suspected acute infarction Prolonged hypotensive states have been postulated as a cause of further extension of an infarct, as has recently been discussed (11).

The present results, regarding patients with both hypotension and signs of congestive heart failure, are more difficult to evaluate as there were only three patients, all with AMI in this group and two were in an apparently preterminal state and died. In the present study no attempt has been made to evaluate prophylactic antiarrhythmic treatment after DC electroconversion (e.g. slow release quinidine) as this treatment was primarily guided by the presence or absence of ventricular arrhythmias.

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HEMODYNAMIC CHANGES AT REST AND DURING EXERCISE IN LONG-TERM CLONIDINE THERAPY OF ESSENTIAL HYPERTENSION

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Abstract. Thirteen men with untreated essential hypertension in WHO stage I, all working, have been studied ambulatorily. Oxygen consumption, heart rate, cardiac output (Cardiogreen) and intraarterial brachial pressure were recorded at rest in supine and sitting position and during steady state work at 300, 600 and 900 kpm/min. The subjects were treated with clonidine as the sole drug for one year and the hemodynamic study was repeated. The reduction in the intraarterial pressure was usually due to decrease in cardiac output which again was due to reduction in heart rate. The calculated total peripheral resistance did not change significantly. The pressure reduction was greatest at rest. During submaximal exercise the differences between the hemodynamic parameters before and after therapy were small.

During the last years the blood pressure (BP) lowering effect of the relatively new antihypertensive drug clonidine has been well documented (2, 3 11 13 16, 18). The hemodynamic mechanism responsible for the *acute* pressure drop (following a transitory rise) has also been well studied in resting supine subjects. Like other antihypertensive drugs interfering with the sympathetic nervous system, the pressure drop induced by acute i.v. or oral administration is associated with a decrease in cardiac output (CO) and little or no effect on the calculated total peripheral resistance (TPR) (12, 14 17 21). However if long term therapy will change this hemodynamic pattern towards reduction in resistance is not known. Such a homeostatic reaction has been documented for the thiazide diuretics (9) and has been suggested for clonidine on the basis of a few observations (20). Since animal experiments have shown that the drug increases the baroreceptor activity (22) such a homeostatic change would not seem unlikely.

The purpose of this work was to study the hemodynamic changes at rest and during exercise induced by one year of treatment with clonidine as the sole drug in subjects with mild essential hypertension.

MATERIAL

The study originally included 15 men, aged 21 to 58 years, with untreated essential hypertension but no other disease with the exception of mild, chronic sinusitis in one subject. All subjects were in WHO stage I. Secondary hypertension was excluded by the usual routine procedures (8). During the time of the study two subjects were lost due to side-effects. The present data concern the 13 men who completed the study. The mean value and S.D. for age, b.wt. and BSA before treatment were 42.6 ± 10.5 y, 80.1 ± 6.4 kg and 1.97 ± 0.9 m². After one year on therapy the b.wt. was 81.7 ± 6.3 kg, but the changes were inconsistent.

METHODS

The subjects were studied hemodynamically during strictly standardized conditions at rest, supine and sitting and during bicycling in steady state at 300, 600 and 900 kpm/min. Oxygen consumption, intraarterial pressure (brachial artery), heart rate (HR) and CO (Cardiogreen) were measured in duplicate in each situation. The methods have been described previously in detail (8), but since the value of hemodynamic studies have been questioned recently (19) it should be pointed out that all subjects knew the investigator well from several ambulant controls before the study. The subjects were informed about the nature and purpose of the study. The investigator did not wear mask and did not look particularly frightening. A mean resting HR of 71 at the first study should not indicate great fear in the subjects—nor the fact that all volunteered for the second study without hesitation and without being submitted to any pressure from the investigator.

All studies were made ambulatorily. After a treatment period of 11–12 months the hemodynamic study was repeated. The difference between the hemodynamic

Table I *Oxygen consumption (VO_2), cardiac index (CI), stroke index (SI) and heart rate (HR) before (I) and after (II) therapy (n = 13)*

	Rest				Work (kpm/min)					
	Supine		Sitting		300		600		900	
	I	II	I	II	I	II	I	II	I	II
VO_2 (ml/min/m²)										
Mean			154.9	138.7	511.5	503.2	773.7	726.0	1101.6	1054.1
S.D.			20.4	12.3	54.6	51.0	67.2	82.1	86.0	143.7
CI (l/min/m²)										
Mean	3.46	3.02	2.87	2.52	5.63	5.48	7.19	6.95	8.22	8.92
S.D	0.35	0.33	0.36	0.19	0.55	0.49	0.63	0.88	1.19	1.33
SI (ml/stroke/m²)										
Mean	48.8	49.8	39.5	39.6	54.8	56.7	57.4	58.9	53.5	58.1
S.D	5.5	8.2	4.4	7.2	7.7	7.7	9.6	9.9	10.0	13.0
HR (beats/min)										
Mean	71.4	61.8	73.1	65.3	104.1	98.0	128.1	119.5	156.3	154.9
S.D	8.5	10.8	10.2	12.4	13.8	13.6	20.3	15.5	19.6	19.5

at the first and second study was tested by Student's *t*-test.

The present study does not include any untreated control group. However it has been documented in a previous work (9) that in similar untreated subjects, the hemodynamic parameters at the restudy after one year were not significantly different from the first.

Treatment

The patients were treated with clonidine (Catapresan® 150 μg tablets). The dose was adjusted according to the [illegible] on the BP and side-effects. The dose varied between 300 and 600 μg/d (mean 383 μg/d). No other [illegible] or any diet restrictions were given. On the day of the second hemodynamic study the patients took their morning dose at 7.00 a.m. The hemodynamic study was performed between 9.00 and 12.00 a.m.

Side-effects

Dry mouth and drowsiness were marked in 11 of the 15 subjects who started the study and intolerable in 2 (one school teacher and one engineer, both nearly fell asleep on job and refused to use the drug [illegible] for 4 weeks). After 2–3 months the side-effects were less disturbing but still prohibited increase in dose in 5 subjects with unsatisfactory BP control. It should be noted that all subjects in this study had no complaints and were free from complications before treatment. Thus, of

Table II *Systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP) and the total peripheral resistance index (TPRI) before (I) and after (II) therapy (n = 13)*

	Rest				Work (kpm/min)					
	Supine		Sitting		300		600		900	
	I	II	I	II	I	II	I	II	I	II
SAP (mmHg)										
Mean	164.5	141.6	178.5	153.1	200.5	183.6	208.8	19[illegible].7	220.3	219.4
S.D	14.1	18.8	17.6	20.8	15.3	23.5	20.0	4.3	19.2	21.9
DAP (mmHg)										
Mean	94.6	82.6	106.3	92.5	108.2	99.3	110.2	100.5	120.0	118.8
S.D	9.0	10.5	11.4	12.1	10.0	13.7	11.7	15.5	11.8	16.7
MAP (mmHg)										
Mean	123.4	104.2	133.0	115.2	145.8	133.0	149.5	136.6	16[illegible]	159.1
S.D	11.0	13.9	12.4	14.4	13.2	19.1	16.0	19.1	13.7	[illegible]
TPRI (dyn sec cm⁻⁵ m²)										
Mean	2 894	2 784	3 741	3 678	2 054	1 952	1 673	1 59[illegible]	1 401	1 4[illegible]
S.D.	460	459	392	485	222	293	217	277	[illegible]	[illegible]

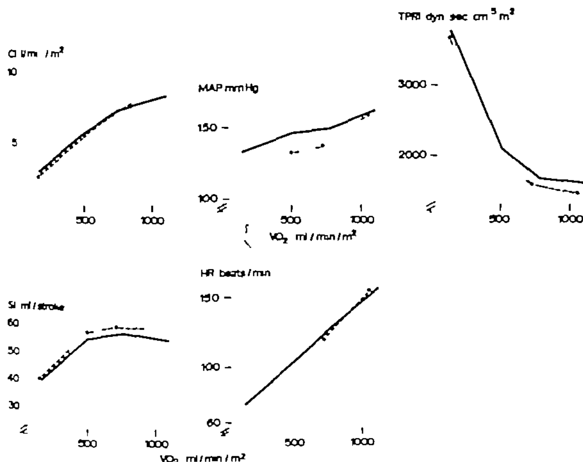


Fig. 1 Hemodynamic changes at rest sitting and during exercise before (——) and after (- -) treatment with clonidine. Mean values. Abbreviations as in Tables I and II.

course, influenced the tolerance of side-effects. An interesting "side-effect" appeared in subject with mild chronic sinusitis and frequent episodes with rhinitis. After clonidine was started his sinusitis was cured and his smell sense, which had been gone for years, returned after 3 months. It is likely that this is due to nasal decongestion, well known effect of clonidine (10).

RESULTS

Casual blood pressure

The casual BP dropped in all subjects during treatment, the mean values from 183/117 before start to 152/99 mmHg at the last routine control. The hemodynamic data are shown in Tables I and II and Fig. 1.

Oxygen consumption

The oxygen consumption (VO_2) at *rest* (sitting) was lower in 11 subjects at the second examination. The mean drop was 10% or 16.2 ml/min/m. The difference between the mean values before and after therapy was almost significant. During *exercise* the VO_2 showed the same tendency as during rest but the changes were not significant.

Cardiac index

At *rest* the cardiac index (CI) dropped in 9 subjects in the supine position and in 12 in the sitting position. The mean drop in the supine position was 0.44 l/min/m or 13% (significant), in the sitting position 0.36 l/min/m² or 12% (significant). In the sitting position 6 subjects had a reduction in CI greater than 10% and the mean values dropped from 2.87 to 2.52 l/min/m². The difference between these mean values is significant. During *muscular exercise* the mean CI after therapy was not significantly different from before.

Heart rate and stroke index

At *rest* the HR was reduced after therapy in 11 subjects in both supine and sitting positions, the mean reductions being 13% and 11% respectively and significant. The difference between the mean values before and after therapy was significant in the supine position. During *muscular exercise* the HR also tended to be lower after therapy but at the highest work load the reduction was only 1%.

The changes in the *stroke index* (SI) were small being at *rest* practically unchanged. During *muscular exercise* the SI tended to be slightly higher after therapy than before.

Arterial pressure

The systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP) were reduced after therapy at rest and during work, the reductions being significant at rest and at the two lowest work levels. The changes in each of these three parameters were rather similar

At *rest* MAP was reduced 16% in the supine and 13% in the sitting position. In the sitting position MAP was reduced 10% or more in 10 subjects Also the differences between the mean values of MAP at rest were significant both in the supine and the sitting positions. During *muscular exercise* the reduction in MAP was less than uring rest. At the 900 kpm work load the MAP higher than before treatment in 4 subjects the mean value decreased only 3 mmHg.

Total peripheral resistance index

Before therapy all subjects had an elevated total peripheral resistance index (TPRI) (at rest sitting $> 2\,700$ dyn sec cm^{-5} m^{2}) The effect of therapy on TPRI was disappointing At *rest supine* the findings were inconsistent (increase in 7 and decrease in 6) and the differences not significant in any body position. At *rest sitting* a reduction in the TPR of at least 10 was seen only in 4 subjects.

During *muscular e ercise* the effect upon the TPRI was also inconsistent A decrease in the TPR of at least 10% at rest sitting and during two work loads was obtained only in 3 patients.

DISCUSSION

This study has shown that long-term therapy with clonidine as the sole drug in subjects with mild essential hypertension induces a moderate reduction in BP in agreement with other workers (6 18). The effect is most marked at rest supine and negligible during maximal work. This could be consistent with the concept that the drug mainly acts upon the central part of the sympathetic nervous system—as demonstrated in various animal preparations (5 7 15). During strenuous muscular exercise local reflexes and regulatory mechanisms may play a much greater role in the control of the CO than during rest. The drug has a marked sedating effect which could be responsible for the reduction in the resting VO_2, HR and CO Similar changes in these parameters have been demonstrated in several acute studies on hypertensive subjects (12, 14 17 21) Thus, the long-term effect does not seem to be different from the acute effect. The present study does not indicate that there is any change in the hemodynamic reaction pattern towards a decrease in resistance and increase in flow even after one year's therapy This is in contrast to what has been suggested by Reubi et al. (20). However most subjects in their study had recei ed a thiazide diuretic in addition to other drugs.

Earlier results in this laboratory of long-term therapy with thiazide diuretics (9) and with α-methyldopa (10) indicate that the hemodynamic response after long-term therapy is not independent on the type of drug. After therapy with α-methyldopa—or clonidine—a decrease in resting HR and CO is characteristic—after therapy with hydrochlorothiazide HR is unchanged, CO slightly and insignificantly reduced, and, *TPRI* significantly decreased Furthermore the pressure reduction during exercise is almost the same as during rest after thiazide diuretics, in contrast to the situation after α-methyldopa or clonidine.

In the resting subject the mean CO was reduced approximately 0 8 l/min. One might speculate in which parts of the body the blood flow was reduced. Studies of the acute effect of clonidine on the regional circulation ha e demonstrated a reduction in skin blood flow (4) and one study also in muscle blood flow (1) Renal blood flow is not decreased (17)

The main conclusion from this and a previous study (10) is that the pressure reduction obtained by long-term treatment with clonidine or α-methyldopa is due to basically similar *hemodynamic* mechanisms—resembling those seen after

short-term use of these drugs (12, 14 17 21). *Comparative studies of the clinical usefulness of* the two drugs generally indicate that they are also rather similar with respect to BP control and side-effects (2, 3 13).

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HEMODYNAMIC CHANGES AT REST AND DURING EXERCISE IN LONG-TERM β-BLOCKER THERAPY OF ESSENTIAL HYPERTENSION

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Abstract. In 10 men with untreated early essential hypertension oxygen consumption and central hemodynamics have been studied before and after one year on alprenolol, 400–800 mg daily. The most important changes (at rest and during exercise) induced by therapy were: marked reduction in heart rate and cardiac index, moderate reduction in arterial blood pressure and no change or increase in calculated total peripheral resistance. No subject demonstrated an unequivocal change in hemodynamic pattern towards reduction in total peripheral resistance without decrease in cardiac output.

During the last few years the β-blockers have been extensively used in therapy of arterial hypertension (5, 13, 20) and become the drug of first choice in treatment of mild and moderate essential hypertension in some centers, mainly because of few side-effects and reasonably good blood pressure (BP) control.

Although studies of the *acute* hemodynamic effect of β-blockers in essential hypertension have shown conflicting results with respect to the changes in the BP (7, 16, 17, 19), it is well established that heart rate (HR) and cardiac output (CO) are decreased. Calculated total peripheral resistance (TPR) is little affected or increased (7, 16, 17, 19). As the predominant hemodynamic disturbance in essential hypertension in subjects above the age of 35 is an increased TPR and normal or low CO (8), *short-term* use of the β-blockers does not correct the cardinal hemodynamic disturbance in established hypertension.

However, it has been documented for the thiazide diuretics, that although the pressure drop induced by *acute* administration is due to a decrease in stroke volume and CO with no decrease in TPR, *long-term* use results in a decrease in TPR and gradual rise in CO. This has been demonstrated both at rest and during exercise (9).

One recent long-term study of the effect of propranolol in hypertension (17) suggests that a similar homeostatic mechanism might operate during chronic therapy with the β-blockers.

The purpose of this work was to study the chronic effect of the β-blocker alprenolol (Aptin®) on the central hemodynamics at rest and during exercise in mild essential hypertension. Studies of the *acute* hemodynamic effect of this β-blocker at rest and during exercise have indicated a possible smaller decrease in CO than with propranolol (6). Long-term studies are lacking.

MATERIAL

The study includes 10 men, aged 30–42 years, with untreated essential hypertension, but no other disease. All subjects were in WHO stage I. At the two first consultations in the investigator's office all had HR > 82 beats/min at rest sitting and diastolic BP (DAP) > 104 mmHg. All 10 completed the study. The mean values and S.D. for age, b.wt. and BSA were 37.1 ± 4.2 y, 80.7 ± 11.6 kg and 1.99 ± 0.11 m². There were no significant changes in b.wt. during the study.

METHODS

The methods are identical to those described in a previous paper about the long-term hemodynamic effect of clonidine (11).

Treatment

The patients received alprenolol (Aptin durettes® 200 mg) at 7.00 a.m. and 5.00 p.m. The daily dose varied from 400 to 800 mg. The dose was adjusted according to the effect on BP aiming at casual sitting DAP of 90–95 mmHg. No other drugs or diet restrictions were given. On the day of the second hemodynamic study the subjects took their morning dose at 7.00 a.m. The hemodynamic study was performed between 9.00 and 11.00 a.m.

Table I. *Oxygen consumption (VO_2), cardiac index (CI), stroke index (SI) and heart rate (HR) before (I) and after (II) therapy (n = 10)*

	Rest				Work (kpm/min)					
	Supine		Sitting		300		600		900	
	I	II	I	II	I	II	I	II	I	II
VO_2 (ml/min/m²)										
Mean			147.9	141.9	511.6	577.6	780.4	788.4	1 103.8	1 133.8
S.D			12.8	11.4	50.5	47.0	55.3	75.2	111 7	114 0
CI (l/min/m²)										
Mean	3.62	3.16	3 19	2.45	5.84	5.28	7 48	6.74	9.05	8.29
S.D	0.44	0.51	0.54	0.28	0.72	0.75	0.73	0.71	0.51	0.81
SI (ml/stroke/m²)										
Mean	46.7	51.1	38.9	37.3	52.7	55.9	55 4	60.4	53.5	59.8
S.D.	4.8	11 1	5 6	4.9	5.1	10.2	4.4	10.7	3.3	10 4
HR (beats/min)										
Mean	78.3	63.0	82.5	64.2	111 7	95.3	136.9	112.8	169.6	139.8
S.D	12.5	7.9	10.5	5.4	13.4	5.7	14.0	8.9	12.5	10.5

Side-effects

One subject (37 years) asked spontaneously after 6 week on therapy if the drug could be responsible for reduction in libido which he had noticed. As disturbances in sexual function are reported to be exceptional with β-blockers he was advised to continue the drug and at the next control 4 weeks later he reported that his libido was back to normal. Two subjects told spontaneously that they felt more "relaxed" during stress situations and did not loose their temper so quickly as before.

RESULTS

The casual BP and HR dropped in all subjects during treatment, the mean values from 171/116 mmHg and 94 beats/min before start to 139/96 mmHg and 70 beats/min at the last routine control. The hemodynamic data and abbreviations are shown in Tables I and II and Fig. 1

Oxygen consumption

There were no significant changes in the VO_2 at *rest* sitting. At the *lowest work* level (300 kpm/min), the VO_2 tended to be higher than before treatment and the mean value increased 13% (significant). At the *two highest work* levels the mean values before and after therapy were almost the same.

Table II. *Systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP) and the total peripheral resistance index (TPRI) before (I) and after (II) therapy (n = 10)*

	Rest				Work (kpm/min)					
	Supine		Sitting		300		600		900	
	I	II	I	II	I	II	I	II	I	II
SAP (mmHg)										
Mean	148 7	130.1	157.0	144.2	179 4	162.3	189 4	169.5	214 6	197.8
S.D.	9.0	17 1	16.9	17.3	16.4	13.7	16.6	15.2	19.5	14 6
DAP (mmHg)										
Mean	92.7	84 1	102.1	95.6	103.3	99.6	106.2	100.5	117.0	113.2
S.D	13.2	12.1	9.8	11.6	10.7	8 6	9.2	11.2	14.9	10 9
MAP (mmHg)										
Mean	115.9	103 7	123.5	115.3	135.6	126.0	137.6	129.9	154.8	144.2
S.D	15.5	13.8	14.8	13.6	13.3	11.5	13.3	11 7	19.5	12.3
TPRI (dyn·sec cm⁻⁵ m²)										
Mean	2 580	2 634	3 136	3 779	1 883	1 944	1 481	1 558	1 377	1 405
S.D.	355	283	426	408	234	358	185	225	236	173

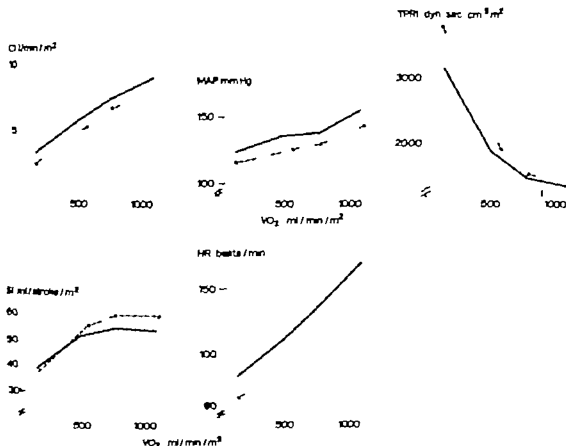


Fig. 1. Hemodynamic changes at rest and during exercise before (——) and after (- -) treatment with alprenolol. Mean values. Abbreviations as in Tables I and II.

Cardiac index

At *rest* the CI dropped in all but one subject in the supine position and in all in the sitting position. The mean drop in the supine position was 0.46 l/min/m² or 13% (significant) and 0.75 l/min/m² or 23% (significant) in the sitting position. In the sitting position the CI was reduced at least 10% in all but one. During *muscular exercise* the CI was reduced in all but one subject at all work levels, the mean reductions being 10% 10% and 8% at the 300, 600 and 900 kpm/min loads, respectively

Heart rate

The greatest changes were seen in this parameter At *rest* HR was reduced in all subjects, mean reduction being 15 and 16 beats/min in the supine and sitting position respectively or 20% At rest sitting HR was reduced more than 10% in all but one. The lowest HR at rest supine after therapy was 52 beats/min (before therapy 64 beats/min). During *muscular exercise* the HR was lower than before therapy in all subjects at all work levels, the mean reduction being about 17% at all work levels.

Stroke index

At *rest supine* mean SI showed an increase of 8% but the changes were not consistent. At *rest sitting* the SI fell in all but one, resulting in a small mean decrease of 4% During *muscular exercise* the changes were inconsistent, but the mean values showed an increase at all work levels of about 7%

Arterial pressure

The BP was moderately reduced in all subjects at rest and during *exercise* The effect was

marked for the SAP At *rest supine* the reductions in SAP DAP and MAP were 13% 9% and 11% respectively At *rest sitting* the reductions in SAP DAP and MAP were 8% 6% and 7% respectively Only in 3 subjects was MAP reduced 10% or more, and in 5 10 mmHg or more. During *exercise* the mean reductions in SAP DAP and MAP were about 10% 5% and 7% respectively

Total peripheral resistance

At *rest supine* the changes in TPRI were inconsistent and the mean value was nearly unchanged (+2% increase). At *rest sitting* however TPRI increased in all but one, and increased more than 10% in 8. The mean increase was 21% (significant). During *muscular excercise* the changes in TPRI were less consistent, the mean values showing an increase of 3% 5% and 2% at the three work levels. A decrease in TPRI of at least 10% during two work loads and at rest supine (but not sitting) was seen in only one subject.

DISCUSSION

The present study has shown that alprenolol in doses of 200–400 mg twice daily induces a mod- but statistically significant drop in BP in ...cts with mild uncomplicated essential hyper ... The mean pressure reduction at rest is the same magnitude as reported by others us- alprenolol in a similar dose (4 18) or propranolol in doses below 1 000 mg daily (2, 12, 17) as the sole drug. In agreement with other short and long term studies on propranolol the reduction in HR is the most marked and most consistent change at rest (3 7 16, 17 19). The reduction is substantial also during exercise. The mean values for HR during exercise after therapy were considerably lower than the mean values in normotensive subjects of similar age previously studied in this laboratory (8). In other words, the HR was abnormally low Low HR values during exercise in subjects treated with propranolol have been reported by others (14 16). At rest supine the marked reduction in HR (20%) was partly compensated by an increase in SI resulting in a smaller reduction in CI (13%). Increase in supine SI after i.v. alprenolol has been reported by others (6). At rest sitting, however the SI did not increase but decreased slightly the net effect being a drastic reduction in CI of 23%—and a 1.5 l/min drop in CO This is the greatest reduction in mean sitting CO induced by any sole antihypertensive drug studied in this laboratory (9 10, 11). In a 10-month study of propranolol in 7 subjects a 1.5 l/min reduction in supine CO was found (3). It is therefore possible that the drop in CO in the sitting position induced by long-term treatment with propranolol might be even greater than the drop seen after long-term treatment with alprenolol.

The VO_2 showed no significant changes at rest. This is similar to results obtained by alprenolol and propranolol in acute studies (6). During exercise, however the VO_2 was higher than before treatment at the lowest work level. It is possible that this could be due to decreased working efficiency in the leg muscles caused by reduction in blood flow However at increasing work loads the VO_2 was no longer significantly different from that before treatment.

The TPRI in the supine position showed insignificant changes and a mean increase of 2% similar to what is reported in propranolol studies (3 17). In the sitting position, however TPRI was significantly increased. The subjects in this study were in their thirties or early forties All but two had an increased TPRI above normal (> 2 700 dyn/sec cm $^{-5}$ m^2) at rest sitting before treatment. All had definitely increased TPRI during work. After one year on alprenolol *no subject* demonstrated an unequivocal change in the central hemodynamics in direction of lower resistance without reduction in blood flow

The β-blockers have been considered particularly useful in early essential hypertension from the point of view that such subjects usually have high CI and HR during rest. It is frequently overlooked, however that also in subjects with early essential hypertension the TPRI is higher than it should have been in relation to the blood flow This is clearly demonstrated during muscular exercise when TPRI is significantly higher than in normotensive controls of the same age (8). Therefore the ideal drug even for a subject with *mild* early essential hypertension should reduce TPRI. However it is reasonable to believe that subjects with a *relatively high CI at rest* and during exercise will *tolerate* reduction in CI better than those who have a low pretreatment CI On the other hand, it is difficult to predict the effect the β-

blockers will have on the BP on the basis of pretreatment CO (1).

With a mean reduction in resting CI of 1.5 l/min in mind, it seems likely that the β-blockers should be used with caution in subjects with low pretreatment CO even if they demonstrate no clinical signs of heart failure.

At present it is frequently debated whether the β-blockers or thiazide diuretics should be the "drug of first choice" in mild and moderate essential hypertension. Although it is beyond the scope of this article to discuss the problem thoroughly it should be kept in mind that long-term therapy with thiazide diuretics decreases TPRI in the majority of subjects with mild or moderate essential hypertension without significant reduction in blood flow (9). From a pathophysiological point of view this is preferable to pressure drop associated with marked reduction in blood flow

Although the β-blockers are usually apparently well tolerated, quite severe and in some series rather frequent, side-effects have been reported (2, 12, 13 20). The reports about the potency of the β-blockers as antihypertensive agents hav also been conflicting (2, 12, 20).

A more satisfactory BP response is obtained with the combination of β-blockers and diuretics (12) or β-blockers and hydralazine (5 1). The latter combination is particularly interesting from a hemodynamic point of view and combines the vasodilating effect of hydralazine with the HR decreasing effect of the β-blockers (15). The future will tell if this combination will be more useful than previous hydralazine regimes.

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INCIDENCE OF PREMATURE BEATS AND ECTOPIC TACHYARRHYTHMIAS AND THEIR POSSIBLE INTERRELATION IN ACUTE MYOCARDIAL INFARCTION

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Abstract. In a prospective study of patients with acute myocardial infarction the incidence of supraventricular and ventricular ectopic beats and tachyarrhythmias has been examined with regard to the site of the infarcts and possible interrelation between the different kinds of dysrhythmias. The material consisted of 356 patients consecutively treated and analysed in Coronary Care Unit (CCU). The mortality rate was 19%. In 68 patients (19%) no ectopic beats or tachycardia were registered. The mortality rate in this group was 13%. In 288 patients with ectopic arrhythmias (81%) the mortality rate was 21% highest for anterior infarcts. The site of the infarcts was without influence on the frequency of the different kinds of dysrhythmia apart from sinus tachycardia and atrial fibrillation, which were most common in cases of anterior infarct. The incidence of ectopic beats and tachycardias was equal to that found in other CCUs. Supraventricular tachycardias including atrial fibrillation did not influence the mortality rate unless they were part of pump failure syndrome. A significantly higher frequency of patients with more than 15% supraventricular ectopic beats was found among patients with supraventricular tachycardia than in patients without. No significant interrelation was found between supraventricular and ventricular ectopic beats. In spite of medical treatment significantly higher frequency of patients with more than 5% ventricular ectopic beats was found in the group with ventricular fibrillation or ventricular tachycardia than in the group without, tendency that was accentuated if the ectopic beats came in salvoes.

Continuous ECG monitoring of patients with acute myocardial infarction (AMI) has revealed a high incidence of premature beats and ectopic tachyarrhythmias, especially during the first few days of the disease (1 4 5 6, 8, 9 11, 12, 13 15).

As accelerated heart rate increases the demand of the myocardium for oxygen t a critical time, and as ectopic tachycardias may precipitate ventricular fibrillation or other serious complications because of reduced cardiac output or may lead to congestive heart failure, a cardinal role of Coronary Care Units (CCU) is the prevention of tachyarrhythmias and their immediate termination if they should occur

In two previous papers (3 4) we ha e given a detailed clinical analysis of the same patient material concerning the influence on the mortality rate of age and sex factors, duration of symptoms before admission, previous ischaemic heart disease, site of infarcts, and conduction disturbances.

In this prospectively planned study we exclusively report an ECG analysis of the incidence of premature beats and ectopic tachyarrhythmias together with an estimation of a possible interrelation between them in patients with AMI consecutively treated in a CCU

MATERIAL AND METHODS

In the period between 24.11.1967 and 1.8.1970, 377 patients were treated because of AMI proven by characteristic history typical changes in ECG and elevated serum enzymes or positive findings at autopsy as described in previous study (4). Twenty-one patients who had cardiac arrest before admission to the CCU are not included in this study The material comprises thereafter 356 patients, 221 males and 135 females, aged between 37 and 89 years.

All patients were kept under constant ECG monitoring until their condition was stable, on average 6 days. The ECG signals were transmitted to constant running 8-channel tape recorder and the recordings were daily analysed for dysrhythmias by at least two of the three authors.

The evaluation of the different kinds of dysrhythmia was based on the presence of premature beats and/or tachyarrhythmias persisting during period of at 100 QRS complexes or during at least 1 min. beats and tachycardias recorded sub finem in

Table I *Site of infarcts and mortality rate in 64 patients with atrial fibrillation following AMI*

Site of myocardial infarction	No. of pats.	No. of deaths
Anterior wall	34	12 (35)
Posterior wall	22	4 (18)
Septum	4	0
Indefinite	4	0
Total	64	16 (25)

of cardiogenic shock and dysrhythmias du to medical treatment ar not included in this study

In the statistic evaluation we ha used the χ^2-test with a significance level of 0.05

Principles of treatment

Generally the medical treatment was pethidine in cases of pain and correction of eventual electrolyte disturbances. In cases of hypoxia oxygen as administered through nasal tube Heart failure was treated by i. furosemide and digoxin. Arterial hypotension as treated ith glucagon, digoxin, isoprenaline or metaradine The main principle for initiating medical antidysrhythmic treatment was that the dysrhythmia persisted mor than 3 min

The treatment of *sinus tachycardia* as always directed at any underlying extracardial cause Sinus tachycardia per se was interpreted as a pump failure rhythm and consequently treated with digoxin

Supraventricular tachycardia (atrial or junctional), atrial flutter and atrial fibrillation er treated with digoxin the heart rate exceeded 100 min and or if ther er of heart failure If the tachycardia could not be ently suppressed, the treatment as supplemented β-blocking agent, by preference practolol, in doses 2–3 mg intravenously up to maximum of 20 mg, occasionall given as drip containing 20 mg in 500 ml isotonic glucose. In few cases ambiline or diphenylhydantoin ere used I efractory or urgent cases synchronized DC cardioversion as undertaken followed by sustained acting quinidine 0.8–1 g daily in tw divided doses.

Supraventricular premature beats exceeding 15 or coming in salvoes ere treated ith digoxin and or practolol. *Ventricular ectopic beats* er treated hen they occurred ith frequency greater than % hen they er multifocal in origin or coming in sal oes, or when they fell in the vulner ble phase For suppression lidocaine was the first drug of choice administered as a bolus dose of 1 mg/kg b.wt. followed by an drip, 20–40 μg kg min. In efractory cases procainamide as used instead of or together ith lidocaine in equal doses Occasionally β-blocking gent and in some cases diphenylhydantoin ere necessary the latter in doses of 100 mg every 5 min up to maximum of 500 mg

Ventricular tachycardia as generally treated as described bove, but if the treatment as not successful within few minutes or the condition led to clinical deterioration, a synchronized DC shock was undertaken

Ventricular fibrillation was always treated with DC shock followed by i.v. infusion of 100 mEq sodium bicarbonate and a lidocaine drip.

Generally all medical treatment as given intravenously If treatment was successful it was continued for at least 24 hours before reduction of the dose was attempted. In cases of reoccurrence of the arrhythmia the dose was immediately increased and a new reduction was not attempted until the arrhythmia had been sufficiently suppressed by oral treatment. In such cases the treatment was continued per os for at least one week befor new attempt as made to reduce or discontinue the treatment.

RESULTS

The total material consisted of 356 patients. 191 (53%) had anterior 131 (37%) posterior 10 (3%) septal infarction, and in 24 cases (7%) the site of the infarction was indefinite as estimated by ECG Sixty-nine patients (19%) died during a 28-day observation period.

In 68 patients (38 males and 30 females) no ectopic beats or tachyarrhythmias were registered. Nine of these died (13%). The cause of death was cardiogenic shock in 5 cases and rupture of the myocardia wall in 4

Ectopic beats and/or tachycardias ere recorded during monitoring in 288 patients (81%), 133 males and 105 females. In this group 60 patients (21%) died.

As determined by ECG or at autopsy the infarcts were located in the anterior wall in 152 patients of whom 41 (27%) died, in the posterior wall in 108 patients of whom 17 (16%) died, in the interventricular septum in 9 patients of whom one died, and in 19 cases the site of the infarct was indefinite one of these patients died.

The cause of death was cardiogenic shock in 33 cases, ventricular fibrillation or asystolia in 15 rupture of the myocardial wall in 7 thromboembolic complications in 4 and congestive heart failure in one case.

Sinus tachycardia was seen in 97 cases (36%), being twice as common in anterior as in posterior wall infarctions. Sinus tachycardia per se did not influence the mortality rate whereas 14 patients out of 17 with simultaneous arterial hypotension died, 10 of these had anterior wall infarctions.

Atrial fibrillation and fluctuation were recorded in 64 patients (22%). The site of the infarcts and the mortality rate are shown in Table I. As seen from this Table the mortality rate was espe

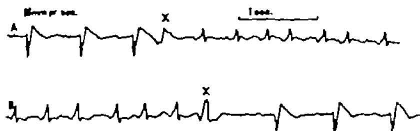


Fig. 1. (*A*) Sinus rhythm, PQ = 0.22 sec. At X ventricular R/T extrasystole initiating atrial fibrillation. Ventricular heart rate 150/min. (*B*) The same patient 15 min later. At X new ventricular extrasystole terminating atrial fibrillation.

cally high in patients with anterior wall infarctions. In this group 7 of the 12 fatal cases had severe hypotension or cardiogenic shock. The mortality rate was independent of the ventricular rate, which was above 100/min in 49 patients. In most cases the arrhythmia was transient, as it only persisted in 5 of the surviving patients.

Simultaneous supraventricular ectopic rhythm was registered in 29 patients and ventricular ectopic rhythm in 42.

Apart from a few cases which were initiated by an extrasystole in a T wave (Fig. 1) no connection was found between atrial fibrillation and other ectopic dysrhythmias.

Isolated atrial fibrillation was seen in only 9 patients. One of these died from pulmonary embolism.

Atrial or junctional tachycardia was registered in 32 patients (9%), equally distributed between anterior and posterior wall infarctions. Only in 3 cases was the tachycardia the sole dysrhythmia, one of these patients died in cardiogenic shock.

Supraventricular ectopic beats (SEB) more than 1% isolated or in connection with other arrhythmias, were seen in 143 patients (40%). The site of the infarcts did not influence the frequency of this arrhythmia (Table II). SEB as isolated phenomenon was seen in 27 patients, 2 of whom died in cardiogenic shock.

Even though SEB was frequently seen together with ventricular dysrhythmias no statistically significant interrelation was found ($p > 0.05$) (Table III), but there was a significantly higher frequency of patients with more than 15% SEB in the group with supraventricular tachycardia than in the group without ($p < 0.05$) (Table IV).

In 116 patients the SEB were unifocal and single, in 23 patients multifocal, coupled or coming in salvoes. These groups are too small for statistical evaluation.

Ventricular ectopic beats (VEB) more than 1% were recorded in 207 patients (58%) equally in anterior and posterior wall infarctions (Table V). The location of the infarcts was without influence on the tendency to development of ventricular tachycardia (VT) or ventricular fibrillation (VF). VEB occurring singly (unifocal or multifocal) were registered in 110 patients of whom 16 (15%) later developed VT or VF. VEB in salvoes (unifocal or multifocal) were seen in 47 patients, of whom 20 (41%) later developed VT or VF.

Ventricular tachycardia occurred in 20 patients (6%) and *ventricular fibrillation* in 25 (7%). The interrelations between VEB and VT and between VEB and VF are shown in Table VI. There was a significantly higher frequency of patients with more than 5% VEB both in the group of patients with VT and in the group with VF ($p < 0.01$).

In 5 cases VF arose without prior VEB and

Table II. *Supraventricular ectopic beats in relation to site of myocardial infarcts in 143 patients*

No. of SEB/ 100 complexes	Site of myocardial infarct (no. of pats.)					
	Anterior wall	Posterior wall	Septum	Indefinite	Total	of 356 pats.
1–5	32	32	3	6	73	21
6–15	15	15	2	1	33	9
15	21	12	0	4	37	10
Total	68	59	5	11	143	40

Table III. *Interrelation between frequency of supraventricular ectopic beats and ventricular ectopic beats in 356 patients with AMI ($p > 0.05$)*

No. of VEB/ 100 complexes	No. of SEB/100 complexes 0–15 (no. of pats.)	> 15 (no. of pats.)	Total no. of pats.
0–5	201	23	224
>5	118	14	132
Total	319	37	356

Table IV. *Interrelation between supraventricular ectopic beats and supraventricular tachycardia in 356 patients with AMI ($p < 0.05$)*

Supraventricular tachycardia > 100/min	SEB/100 complexes 0–15 (no. of pats.)	> 15 (no. of pats.)	Total no. of pats.
Tachycardia	23	9	32
No tachycardia	296	28	324
Total	319	37	356

in one case after a period of 1–5% VEB. In the remaining 19 cases VF occurred after a period more than 15% VEB. In 16 of these patients as preceded by a short period of VT. patients (60%) with VF and 4 (20%) VT succumbed immediately or during the wing observation period, the latter 4 all due to cardiogenic shock.

DISCUSSION

Since heart rate is an important determinant of oxygen consumption, tachycardia of any type is undesirable, especially when the myocardium is under strain, as is the case in the period of an AMI.

Antiarrhythmic drugs, when used in adequate doses, have shown a significant effect upon the incidence of ectopic beats and serious tachyarrhythmias (5, 7, 10, 13, 14), so antiarrhythmic treatment is a routine procedure which no CCU dare leave undone. Therefore, when evaluating materials from CCUs dealing with the incidence and development of ectopic arrhythmias, it must be emphasized that most of the patients have been under medical antiarrhythmic treatment, which was also the case in this study.

The incidence of ectopic beats and tachyarrhythmias in this investigation is of the same order of magnitude as found in many other CCUs (1, 6, 8, 9, 12, 13). Apart from atrial fibrillation we did not demonstrate any connection between the incidence of ectopic rhythms and the site of the myocardial infarcts as judged by ECG, which is in accordance with Mogensen's findings (13), whereas Jewitt et al. (8) found a higher frequency of supraventricular arrhythmias in anterior infarctions.

This investigation did not show any interrelation between SEB and ventricular tachyarrhythmias and has demonstrated that SEB and supraventricular tachycardias including atrial fibrillation—unless they were part of a pump failure—were without influence on the mortality rate. This was also the experience of Kloss and Haywood (9) and Jewitt et al. (8) but is in contrast to Stock et al. (16), who found a mortality rate of 53% in patients with supraventricular arrhythmias, which was higher than the 30% for the whole series.

On the other hand we have found a significant interrelation between supraventricular tachycardia and SEB exceeding 15%.

The presence of VEB in a number of more than 5% caused, in spite of medical treatment, a significantly higher risk of VT and VF and

Table V. *Ventricular ectopic beats in relation to site of myocardial infarcts in 207 patients*

No. of VEB/ 100 complexes	Site of myocardial infarction (no. of pats.) Anterior aII	Posterior aII	Septum	Indefinite	Total	% of 356 pats.
1–5	44	41	3	2	90	43
6–15	29	18	3	5	55	27
>15	31	23	4	4	62	30
Total	104	82	10	11	207	100

Table VI. *Interrelation between number of ventricular ectopic beats and ventricular tachycardia or ventricular fibrillation in 356 patients with AMI ($p < 0.01$)*

	VEB/100 complexes		
	0–5 (no. of pats.)	> 5 (no. of pats.)	Total no. of pats.
With VT	11	25	36
Without VT	228	92	320
With VF	6	19	25
Without VF	233	98	331
Total	239	117	356

than a significant increase of the mortality and this tendency was accentuated if the ectopic beats came in salvoes, regardless of whether they were unifocal or multifocal, which is in accordance with the findings of Mogensen (13).

The incidence and survival rate of VF and VT in our series was similar to the findings of Bennett and Pentecost (2), Lawrie et al. (11) and Mogensen (13), whereas Sloman et al. (15) re... that the incidence of VT was 15% and of ... 23% with a mortality rate of 53% and 78% ...tively in 126 coronary patients.

The results of this study fit very well with the principles of medical treatment undertaken, namely that SEB more than 15% and VEB more than 5% should be treated, as they involve a high risk of serious tachycardias, and that the treatment of VEB, when coming in salvoes, should be initiated immediately.

On the other hand one must admit that, although much has been accomplished in the past ... to reduce mortality due to arrhythmias in AMI, we still meet cases refractory to medical treatment and cases of VF apparently occurring without warning. In a recent study Bennett and Pentecost (2) pointed out that 26% of their patients (in our material 20%) experienced their first cardiac arrest without or after less than ... min of warning and that no drug regimen currently available has sufficient therapeutic value ... low enough toxicity to justify its routine use. This tells us that we must still search for more antiarrhythmic drugs and still be on the ... for new signs predicting imminent cardiac arrest.

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LETTERS TO THE EDITOR

LEVODOPA AS A TREATMENT OF OBESITY

Dear Sir

After introduction of levodopa in the treatment of Parkinson's disease, nausea and vomiting have been reported as conspicuous side-effects (2, 5). This, in combination with our observation of considerable weight loss in some patients treated with levodopa for Parkinsonism, has led us to make a trial of the effect of an emetic agent on body weight in obese patients without neurological disorders.

The study was double-blind and randomized, and as criterion of effect we chose the change in b. wt. after 6 months treatment. Thirty-seven patients received levodopa and 21 served as controls. There were no differences in age, sex or initial b.wt. The latter varied between 74 and 12 kg. The criterion for admission was over weight exceeding 20% (4). The initial dosage was 400 mg levodopa (supplied by Astra) three times a day gradually increasing until nausea and possibly vomiting. When these side-effects occurred, the dosage was diminished. Maximal dosage was 4.8 g levodopa daily No dietary or other treat ment was given.

There were no statistically significant differences in weight alteration between the levodopa and placebo groups after 6 months or at any time during the trial. In the levodopa group 47% (95% confidence limits 30–65%) lost more than 1 kg after 6 months against 33% (95% confidence limits 15–57%) in the control group. The overlapping was considerable. In both groups there were individual cases with weight loss of up to 13 kg, but average weights changed only a few hundred grams. In contrast to the placebo patients practically all members of the levodopa group complained periodically of nausea and vomiting. Nevertheless they stated that they were able to eat their usual meals.

It is remarkable that, in obesity daily nausea and even omiting is compatible with an unaltered food intake and an unaffected b wt Our knowledge of the effect of levodopa on neurologically normal persons is very limited (1 3) It is, therefore, worth noticing that the other side-effects of levodopa, known from its use in the treatment of Parkinson s disease—hyperkinesia, arterial hypotension, confusion and insomnia—were not observed in any of our obese pa tients. A drug with effective hunger-reducing qualities is still lacking in the treatment of obesity

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Gentlemen,

In paper by Haqvin Malmros et al. entitled "Coagulation defects and atherosclerosis induced in rabbits by diet containing medium chain triglycerides (MCT)" and published in Acta med. scand. 192: 201, 1972, the authors described number of changes observed in rabbits fed highly purified diet containing 20% of triglycerides composed mainly of C 8 and C 10 fatty acids. Their observations of hypercholesterolemia and liver cirrhosis seem to be contrary to those of many other investigators.

In our laboratory rats were fed ten dietary fats at level of 20% in a life time study: those given MCT had the longest average life span. Cynomolgus monkeys thrived when fed MCT as their main dietary fat for two years in a filled milk. It first seemed that the results of the Swedish group were due to the fact that their diet contained 20% of fat, which is probably unphysiological for rabbits. However it seems more likely that their results were consequence of linoleic acid deficiency. Their triglyceride mixture contained only 1% of linoleic acid, which gav dietary level of only 0.2%. It is generally thought that the minimum dietary level should be 1% of calories, with the optimum at 3 or 4% of calories.

Therefore, the studies of Malmros et al. are more contribution to the problem of essential fatty acid deficiency rather than to that of MCT metabolism, per se. Malmros himself emphasized that his experiments were incomplete because of the lack of control animals given sufficient essential fatty acids. It is premature to ascribe the observed changes to MCT. Available evidence suggests that the opposite is the case when enough essential fatty acid is supplied.

Very truly yours,
Hans Kaunitz, M.D. Clinical Professor
College of Physicians and Surgeons of
Columbia University New York

Sir

In his letter to the Editor Professor Kaunitz questioned whether the coagulation defects and the atherosclerotic lesions in our experiments on rabbits fed 20% MCT might not be due to essential f tty acid deficiency. This possibility naturally sounds quite feasible since the triglyceride mixture contained only 1% linoleic acid. As matter of fact w discussed the possibility of elation between deficiency of essential fatty acids and the development of atherosclerosis as early as 1959 in an article in The Lancet (1). W had found that mild to moderate hypercholesterolaemia could be induced in rabbits by semisynthetic diet completely free from fat. In animals in which the hypercholesterolaemia was most pronounced, postmortem examination revealed moderate atheromatous plaques in the aorta. W also found that incorporation of 8% corn oil in the semisynthetic diet was promptly followed by a fall in the plasma cholesterol.

Using the same method later systematically studied the hypercholesterolaemic and atherogenic effect of different saturated fatty acids in the form of triglycerides (2). The results of these experiments varied with the type of fatty acid included in the experimental diet. Lauric acid (C12) and myristic acid (C14) induced fairly severe hypercholesterolaemia and pronounced atherosclerotic lesions. Palmitic acid (C16) produced a moderate rise in the plasma cholesterol. Stearic acid (C18) and butyric acid (C4) had practically no enhancing effect on the plasma cholesterol. In all these experiments with different fatty acids pur preparations were used which were practically free from linoleic acid and other polyunsaturated acids. Thus, since some fatty acids considerably raised the plasma cholesterol, while others had little or no such effect, and since the same linoleic-acid-free basic diet was used in all previous experiments as well as in the last experiment with MCT the differences in the results can hardly be explained by essential fatty acid deficiency alone. On the other hand, one might very ell imagine that some of the fatty acids studied hav notable cholesterol-raising effect, while others hav but little, if any such effect.

In our earlier experiments w did not notice any signs of disturbance of the blood coagulation. It therefore appears less likely that the coagulation defects we found in rabbits fed diet containing 20% MCT can be explained entirely by essential fatty acid deficiency and that they would hav nothing to do with the fatty acids in MCT. Neither is it probable that the large doses of fat per se would be the cause of the changes observed. For in long-term experiments with other fatty substances, such as corn oil and sunflower seed oil, w hav used 20% fat and found no injurious effect at all.

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CARDIAC ARREST AS A PRESENTATION OF MÜNCHHAUSEN SYNDROME

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Abstract This paper describes two male patients presenting with cardiac arrest who were later found to be free of ischaemic heart disease. It is suggested that this is a further form of presentation of the Munchhausen syndrome as originally described by Asher in 1951.

In his description of the Münchhausen syndrome in 1951 Asher (1) described three varieties of presentation. 1) the acute abdominal type (laparatomophilia migrans), 2) the haemorrhagic type (haemorrhagic histrionica), and 3) the neurological type (neurological diabolica).

Even though the types of patients described by Asher were recognized prior to his article, he stimulated far more discussion and case reports, apocryphal or otherwise, than had previously appeared. These have included such wide ranging diagnoses as placenta praevia (6), porphyria (8), meningitis (2) and urethral bleeding (5). Chest pain suggestive of either myocardial infarction or pulmonary embolism has also been described by various authors (2, 3 4 7).

We report two cases of cardiac arrest which we consider to be an unusual presentation of the Münchhausen syndrome.

CASE REPORTS

Case 1

The patient, a 40-year-old man, walked onto a ward at the Central Middlesex Hospital, London, on Sept 14th 1968 demanding to visit a relative. By the time that the nurse had returned to inform the man that this relative could not be found the patient was sitting outside the ward complaining of central chest pain radiating down both arms. He then collapsed onto the floor and, according to the nurse, became blue and pulseless. The resuscitation team found that the patient was still "unconscious" and that external cardiac massage had been initiated. The patient was transferred to the Coronary Care Unit where an ECG was normal, BP 140/100 mmHg, pulse 64/min and regular. In view of the fact that the patient was supposedly unconscious and had a full bladder, preparations were made for urethral catheterization. At this point he recovered consciousness enough to protest and complain about his chest pain. His description of the pain and the appropriate mimicry of its vice-like nature were so totally persuasive that he was given an i.m. injection of 10 mg morphine plus 5 mg Fentazin.

Amongst the patient's belongings a card was found with visiting times from the Whipps Cross Hospital. Suspicions were aroused enough to make enquiries. The patient's movements in the few days prior to this admission had been as follows: 11.9.68–12.9.68 Whipps Cross Hospital complaining of chest pain with the diagnosis of "probable myocardial infarction". Taken own discharge. 12.9.68–13.9.68 St. Stephens Hospital, Fulham. The diagnosis on this occasion being "tuinal overdose and myocardial infarction". Taken own discharge. 13.9.68–14.9.68 Willesden General Hospital with "probable myocardial infarction". Taken own discharge. 14.9.68 present admission.

The patient made an uneventful recovery and was well enough after a night's sleep to sit up knitting a scarf and propose marriage to the house physician (female). Arrangements were made for a psychiatrist to see the patient but he took his own discharge on the evening of Sept. 15th

Case 2

The patient, a 34-year-old man, was brought to the Casualty Department of the Central Middlesex Hospital on Dec. 4th 1968 apparently having collapsed in the street with chest pain radiating to his neck and arms, he was groaning with pain. The pulse was 80/min and regular BP 140/90 mmHg; the remainder of the physical examination was normal apart from a cut-down scar at the right ankle (reputedly caused by his dropping a razor blade), and several venepuncture marks in the antecubital fossae. In the presence of a junior nurse the patient stopped breathing and appeared to lose consciousness; cardiac massage was commenced. The resuscitation team arrived to find him recovered. ECG showed no significant

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normality Suspicions were aroused by the inconsistent clinical picture On further questioning the patient revealed some of his previous movements: earlier in December he had been admitted to the Cambridge Military Hospital, Aldershot, with a similar story Münchhausen syndrome had been diagnosed and he had discharged himself having refused psychiatric help. With these facts in mind he was discharged from Casualty

The patient reappeared on April 1st 1969 again complaining of chest pain. He was seen by a medical registrar who recognized him and sent him away The following day he staged a cardiac arrest in the hospital corridor and was taken to the ward only to discharge himself next morning He has been seen again by one of the authors (W.J.W.M.) at the Middlesex Hospital in 1970 having swallowed a coin, and at University College Hospital in 1972 having feigned cardiac arrest. At the age of 6 years he had started swallowing coins, apparently he could hold them in his mouth to help a stammer He seems to have become an established Münchhausen in his 20s and has since been hospitalized in many parts of the British Isles under eleven different names.

COMMENT

We feel that it is necessary to describe these two cases and bring to our colleagues attention what we consider to be yet another presentation of the Münchhausen syndrome. Should this be more aptly entitled asystolica diabolica or asystolic praecox?

The enthusiasm of hospital cardiac arrest teams lends itself to abuse by the cardiac Münchhausen: the nature of the emergency often leaves little time for close assessment of the patient. The danger to these patients is not inconsiderable: in a recent escapade of case _, respiratory failure was induced by i.v. morphine given for his supposed myocardial infarction fortunately this was readily reversed.

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LEUCOERYTHROBLASTIC ANAEMIA, THROMBOCYTOPENIA AND EOSINOPHILIA IN ASSOCIATION WITH BRONCHIAL ADENOCARCINOMA IN A YOUNG WOMAN

A Case Report

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Abstract. A case of rapidly fatal bronchial adenocarcinoma in an 18-year-old woman is reported. Remarkable features of the case included early metastasis to bone and development of leucoerythroblastic anaemia with thrombocytopenia and eosinophilia.

Blood dyscrasias have been reported as a result of metastatic bone marrow infiltration. Leucoerythroblastic anaemia, in which nucleated red blood cells and immature white blood cells are seen in the peripheral blood, and in which thrombocytopenia and haemorrhage are common, as well as the so-called microangiopathic type of haemolytic anaemia have been described.

Several authors (2, 4, 6) have reported cases in association with gastrointestinal tract neoplasms and one (3) in association with prostatic cancer. Few cases have been reported in which the primary site was a pulmonary tumour.

We here describe an unusual case in which leucoerythroblastic anaemia with thrombocytopenia and haemorrhage occurred in an 18-year old woman with a bronchical adenocarcinoma.

CASE REPORT

The patient, an 18-year-old white woman, presented at the beginning of Sept. 1971 with symptoms of cystitis and low back pain. She had previously been completely well. Physical examination was unremarkable and laboratory investigations revealed Hb 13.2 g/100 ml, WBC 18 500/mm³ and ESR 32 mm in the first hour. Urine sediment contained clusters of white blood cells. Initial treatment with sulphonamide and analgetics afforded no symptomatic relief.

On Sept. 15 1971, she was admitted to the Gynaecological Department because of abdominal pain. Physical examination revealed tenderness in the right iliac fossa region and laparoscopy was performed. Right-sided salpingitis was found. Postoperatively she developed massive epistaxis and bled approximately 1 700 ml. She lost consciousness. Laboratory tests showed thrombocytopenia (platelet count 35 000/mm³) and leucocytosis (WBC 25 000/mm³). Liver function was abnormal (total bilirubin 1.3 mg/100 ml, alkaline phosphatase 12.3 Bodansky-Lagan units and S-GOT and S-GPT 100 and 150 Karmen-Wroblewski units, respectively). Chest and abdomen X rays, lumbar puncture and echoencephalography were unremarkable. An attempted bone marrow aspiration failed.

On Oct. 1 the patient was transferred, still unconscious, to the Medical Department. At the time of transfer physical examination revealed a comatose patient suffering episodical, symmetrical convulsions of the extremities. Petechial haemorrhages and ecchymoses were abundant and she was bleeding from the nose. Laboratory investigation showed Hb 8.4 g/100 ml, WBC 23 500/mm³. The differential count showed myeloblasts 0.5%, promyelocytes 2.5, myelocytes 3, metamyelocytes 4, band forms 18, segmented neutrophils 38.5, eosinophils 10.5, basophils 1.0, lymphocytes 20.5 and monocytes 3.5%, and nucleated erythrocytes totalled 3% of the WBC. The platelet count was 32 000/mm³ and ESR 18 mm in the first hour. Other tests revealed the serum creatinine to be raised to 1.5 mg/100 ml, total bilirubin had increased to 2.5 mg/100 ml, alkaline phosphatase was 4.1 B.L. units and S-GOT and S-GPT 300 and 70 K.W. units, respectively.

An iliac crest biopsy revealed infiltration of the bone marrow by highly anaplastic cells whose site of origin could not be ascertained. A chest X-ray on Oct. 1 showed many rounded parenchymal lesions, presumed to be metastatic deposits. Treatment hereafter was symptomatic and the patient died on Oct. 9, three weeks from the date of onset of symptoms, without regaining consciousness.

At autopsy the primary tumour was found to be a small growth (6 × 3 mm) in the left inferior lobe bronchus. Metastases were found in the regional lymph nodes, liver, spleen, ovaries and vertebral bone marrow. Haemorrhage



Fig. 1 Section of left inferior lobe of bronchus, showing infiltration by poorly differentiated adenocarcinoma. Van Gieson × 39



Fig. 3 Vertebral bone marrow with invading tumour between bone trabeculae. Van Gieson × 39

and necrosis were seen in many deposits. No extramedullary haematopoesis could be identified. The brain was surprisingly normal. There was mild cerebral oedema but no evidence of metastases or haemorrhage.

Histologically the primary tumour was poorly differentiated adenocarcinoma (Figs. 1 and). It was located in the mucosa and submucosa of the bronchus as well as in the adjacent lymph vessels (lymphangitis carcinomatosa).

DISCUSSION

The occurrence of a poorly differentiated bronchial adenocarcinoma in a young woman is un- . The rapid downhill course was remarkable was caused by metastatic infiltration of the bone marrow and consequent blood dyscrasia.

Many surveys of large numbers of cases of pulmonary neoplasms have been reported. Of 866 cases reviewed by Bryson and Spencer (1), 743 (86.5 %) occurred in males. Five per cent of these growths were adenocarcinomata most often associated with metastases. Walther (8) examined 2 080 cases of cancer of the lung at autopsy and found metastases in the liver in 35 % bone marrow in 20 % and spleen in 3 % Hanbury (5) reported three cases of lung cancer in young persons, two in males (aged 16 and 22, the former with an oat cell carcinoma, the latter with a poorly differentiated mixed cell growth) and the other in a 12-year-old girl (undifferentiated tumour) All cases were rapidly fatal (survival time 7–9 /₂ months) and one of them developed leucocytosis.

Anaemia in malignant disease may be caused by invasion of the bone marrow or by bleeding associated with the primary or secondary tumours. Bleeding from the gastrointestinal or genitourinary tracts may be aggravated by thrombocytopenia caused by marrow invasion. Vaughan (7) reported that only a relatively small area of marrow needs to be invaded to produce anaemia or blood dyscrasia. The unusual features of the present case are the early haematological changes which are rare in association with lung cancer



Fig. 2. Tumour-filled vessels in the submucosal layer of the bronchus. Van Gieson × 74

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MYELOID METAPLASIA IN DISSEMINATED VASCULAR DISEASE

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Abstract. Myeloid metaplasia has been demonstrated in patient after the onset of fulminant obliterative thromboangiitis and in another patient prior to the onset of systemic sclerosis. In regard to the former patient it was suggested that the bone marrow failure had resulted from thrombotic obliteration of the nutrient vessels of the bone marrow The favourable effect of immunosuppressive treatment upon the peripheral circulation, wound-healing and anaemia in this patient suggested an immune pathogenetic mechanism ith respect to the vessel damage. In the latter patient no relationship was pparent between the myeloid metaplasia and the systemic sclerosis, which became manifest 10 years later Impaired extramedullar haematopoiesis and further progress of the osteomyelosclerosis by reason of arterial involvement in the spleen and bone marrow may have contributed to rapid progression of the anaemia during the last months of the patient's life.

Myeloid metaplasia is a characteristic part of the clinical picture of myelofibrosis. Most instances of myelofibrosis are idiopathic (agnogenic myeloid metaplasia), with the cause of bone marrow failure still unknown.

The compensatory formation of haematopoietic tissue in extramedullary sites arising from the de struction or replacement of bone marrow has been used to explain the myeloid metaplasia, sec ondary to tuberculosis (3), carcinomatous meta stases (6), multiple myeloma (2), malignant lymphoma (10), radiation (1) and benzene exposure (12). Moreover secondary myeloid metaplasia has been observable in a wide variety of other diseases with bone marrow engagement (5 13). A marked proliferation of abnormal marrow cells, or necrobiosis of the cells induced by toxins (13) or allergy (4), are known mechanisms of bone marrow destruction in these cases.

We wish to draw attention to disturbed arterial circulation as the possible cause of bone marrow failure and secondary myeloid metaplasia by re porting on two patients with this clinical entity

CASE REPORTS

Case 1

In 1962 the patient, 36-year-old man, observed cyanosis on the fingers on exposure to cold. He had been heavy smoker since the age of 20. Within three years his symptoms gradually became orse, spreading to the feet and toes, and causing intermittent claudication In 1966 gangrenotic ulcer appeared on the great toe of his left foot. He was admitted to hospital in May 1967 The left toe was gangrenotic and the other toes had bluish discoloration. The fingers and the toes of the right foot were cold and the skin on the distal parts of the extremities was dry and cyanotic. Some bluish-red nodules were pparent in the skin of both calves. The peripheral pulses in the feet were absent. The spleen was palpable 4 to 5 cm below the left costal margin. The liver was not enlarged. Ther was no lymphadenopathy Arteriography disclosed an obliteration of the distal part of the right femoral artery: the left side was normal. Occlusion of the left radial artery was demonstrated in the upper extremities.

ESR was 2 mm/h, haematocrit 51% Hb 16.7 g/100 ml, leucocytes 7 100/mm³ with 65% neutrophils, 9% band forms and 16% lymphocytes. Tests for rheumatoid factor were negative and no LE cells were found. The total serum protein concentration was 7 g/100 ml, with normal electrophoretic and immunoelectrophoretic separa tion patterns. No cryoglobulins were detectable. A biopsy specimen from one of the calf skin nodules showed phlebothrombosis and obliteration of middle-sized artery by an organizing thrombus. In the arterial all and adjacent to the thrombus wer observed round cells and proliferation of fibroblasts. The histological finding was consistent ith later stage of thromboangiitis obliterans, although no giant cells ere pparent. Some clusters of lymphocytes were seen between the muscle fibres in calf muscle biopsy specimen.

During the next three years continuous deterioration occurred in the circulation of all extremities, desp porary relief following lumbar and thectomy Amputation of the right leg and



Fig. 1. Case 1. Advanced thromboangiitis obliterans and myeloid metaplasia in a 41-year-old man. The size of the spleen is indicated by the drawn curve. Note the healed scar between the shoulders.

became necessary. A skin graft, applied on the thoraco-dorsal sympathectomy operation wound, which had been *open for one year, was rejected and a new transplant was applied in Aug. 1971.*

On Feb. 22, 1971 the patient was admitted to hospital by reason of a respiratory infection. The remaining fingers were then cold and cyanotic. The abdomen was rounded; the liver was felt 6 cm below the costal margin and had a *smooth and non-tender surface.* The spleen was markedly enlarged (Fig. 1) and extended to the lower abdomen below the umbilical region. Laboratory studies gave the following findings: ESR 76–6 mm/h, Hb 10.8 g/100 ml, haematocrit 35%, erythrocytes 3.70 mill/mm³, MCH 30 pg, and normal serum iron and TIBC concentrations. The leucocyte count was 9 300/mm³ with an increased number of band forms. The platelet count was normal. In the peripheral blood marked anisocytosis was visible with ovalocytes and poikilocytes in particular. Normoblasts were present. The reticulocyte count was ...1%. The leucocyte alkaline phosphatase score was 145. Bone marrow aspiration attempts from the sternum yielded dry taps, but successful aspiration from the posterior

iliac crest showed cell-rich marrow with normoblastic erythropoiesis and normal granulocytopoiesis. A search for the Ph chromosome was negative. An aspiration biopsy specimen from the sp een disclosed myeloid metaplasia. Subsequently marrow aspirations from othe parts of the iliac crest yielded dry taps. It was accordingly assumed that the patient had patchy myelosclerosis, with scattered active haematopoetic foci of the marrow and secondary myeloid metaplasia f the spleen and liver. The Coombs test was positive with anti-human-globulin serum, and with specific anti-IgM, and anti-C'3 sera. The acid-haemolysis test for the PNH syndrome was negative. Quantitative estimation f the serum immunoglobulins showed IgG 640, IgA 70 and IgM 80 mg/100 ml. The C'3 content f the serum was within the normal range.

Tests for rheumatoid factor LE cells and cold agglutinins ere again negative.

Splenoportovenography revealed open, but slightly widened splenic and portal eins. No bone changes were roentgenologically detectable.

Prednisolone treatment, 20 mg day was begun on March ., 1972, and has since been continued t a daily dosage of 10–20 mg. This treatment yielded good response, with relief of coldness and pains in the fingers and toes, and rapid healing of the dorsal operation wound (Fig. 1). ESR fell to 2 mm/h and Hb rose to 14.8 g/100 ml; they hav remained at these levels Coombs test became negative. The size of the spleen has remained unchanged. The prednisolone dosage could be reduced hen azathioprine (Imurel®) at rate of 200 mg day was added to the regimen. At this time skin, muscle and bone trephine biopsy was performed from the anterior iliac crest. Histological examination of the specimens revealed atrophic skin, normal muscle tissue and cell-rich bone marrow Unfortunately no arteries were found in these specimens. The biopsy wound healed very slowly and as complicated by suppurative fistulation and an osteomyelitic process An increase in the prednisolone dosage to 30 mg day in combination with antibiotic therapy markedly accelerated the healing process. In view of this complication no further bone biopsies have since been performed for confirmation of the diagnosis of osteomyelosclerosis. The immunosuppressive treatment has led to sustained relief of the symptoms and to retardation of the progress of disease

Case 2

A female teacher born in 1913 had suffered from chronic pyelonephritis since 1939. In 1946 she had partial paresis of the left arm. A carotis angiography revealed an aneurysm, but no operation as performed I the same year she was examined by reason of anaemia. The spleen was enlarged and cytological aspiration specimen from the organ displayed extramedullary haematopoiesis She was persistently anaemic, and blood transfusions were given several times during the succeeding years. The leukocyte count aried between 000 and 5 000/mm³ Abnormal red cells, normoblasts and myelocytes ere present in the peripheral blood Bone marrow aspiration attempts yielded dry taps, and in 1958 myeloid metaplasia of the liver as diagnosed

At the beginning of 1959 she complained of cold hands and feet. It was first assumed that she had Raynaud' disease, but gradually the skin of the legs and arms became thick and coarse and the pigmentation increased. Three skin biopsies performed in 1961 and 1962 indicated progressiv scleroderma Congestiv heart failure developed in 1961 Subsequently the enal function deteriorated and she died of uraemia in Dec 196.

The post-mortem xamination macroscopicall revealed markedly enlarged spleen (750 g), somewhat enlarged liver (1 700 g), and small kidney (lef 80 g right 125 g). The histological examination confirmed the clinical features of osteomyelofibrosis ith extramedullary haematopoiesis, and of chronic pyelonephritis V scular lesions ere particularly observable in the spleen d in the kidneys. The small arteries and arteriols had markedly thickened enal alls, probably ttributable to h scleroderma or secondary to the renal disease Staining for amyloid was negative.

DISCUSSION

The control of blood cell production and release into the blood stream is carefully regulated for the maintenance of normal cell alues The major factor that controls red cell production is the tissue tension of oxygen. The stimulatory effect of chronic anoxia on erythropoiesis is shown by the secondary polycythaemia. In obliterati e thromboangiitis chronically impaired arterial circulation occurs in the limbs affected. In fulminant cases the tissue anoxia possibly stimulates red cell production. Anaemia is in fact an uncommon feature of Buerger's disease as was shown with patient 1 in whom normal or increased haematocrit values were observable during the first stage of the disease. Thrombotic invol ement of centrally located arteries, such as the mesenteric, coronary and renal arteries, are uncommon manifestations of Buerger's disease (11). To date, involvement of the nutrient arteries of the bone marrow has not been observed in this disease. In fulminant cases with widespread vascular damage thrombotic obliteration of the marrow vessels may occur this might give rise to bone marrow failure. An early compensatory myeloid metaplasia may then result, following failure of the marrow to respond to the increased demand of red cell production by the chronic peripheral tissue anoxia.

Thromboangiitis obliterans is manifested by segmentally located vascular lesions (11). If the n trient arteries of the bone marrow can be involved in the disease, it seems reason-

patchy osteomyelosclerosis will result, with scattered foci of marrow tissue acting normally Such a pathogenetic mechanism well fits the clinical features in regard to patient 1 Marrow aspirations occasionally yielded cell-rich specimens no more than a few cm from sites of dry tap aspirations.

Experimentally secondary myeloid metaplasia has been induced by the production of multiple marrow vessel infarctions (8) but not by operative disruption of the nutrient vessels (7). These observations may support the hypothesis of bone marrow failure arising from impaired arterial supply as a possible pathogenetic mechanism of myeloid metaplasia.

No general acceptance has been found for any aetiology of Buerger's disease. The most attractive theory is that this disease is a syndrome with more than one causative factor The marked effect of immunosuppressive treatment upon wound-healing, the peripheral manifestations of the disease, and the anaemia in case 1 suggest an immune pathogenetic mechanism.

Recently it has been speculated whether destruction of the marrow sinusoidal microcirculation by an immune mechanism may induce human aplastic anaemia (9). Aplastic anaemia osteomyelosclerosis, however have different and histological features. It may be that [illegible] ment of nutrient vessels in Buerger's may lead to proliferation of the fibroblasts the bone cavity resulting in myelofibrosis, for instance as in disseminated myocardial fibrosis arising from diffuse coronary arteritis or sclerosis.

In patient 2 the myeloid metaplasia preceded the manifestations of disseminated sclerosis by about 10 years. When the symptoms arising from this disease became worse the anaemia progressed rapidly It must be considered that in this case the osteomyelofibrosis was already generalized at the onset of the scleroderma arteritis, as indicated by the repeated dry taps many years before. Consequently impaired extramedullary haemopoiesis attributable to invol vement of the spleen arteries seemed more reasonable than additional bone marrow failure arising from impaired circulation. At autopsy splenic arterial lesions were demonstrated, but no clear relationship was apparent between them and the myeloid metaplasia and the course of the disease. In addition, this patient had markedly impaired renal function, this undoubtedly worsened the anaemia.

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A HEREDITARY RENAL DISEASE WITH CLINICAL AND HISTOLOGICAL PICTURE AS IN CHRONIC GLOMERULONEPHRITIS

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Abstract. A family with hereditary disease with the following characteristics is described: early proteinuria, severe hypertension and progressing renal failure, which as a rule reached the final stage at an age of [illegible] years. Women have been attacked as often as men. Deafness or other defects were not seen. The inheritance was apparently dominant. Histologically changes were found similar to those in chronic glomerulonephritis with synechia, hypercellularity, mesangial thickening, hyalinization of glomeruli and [illegible] vessel changes.

During recent years hereditary renal diseases have attracted increasing interest, mostly because of the improvements in the treatment of renal failure. In the [illegible], we present a family with hereditary [illegible] in several respects [illegible] described.

[illegible] 0–1 leucocytes, 3 erythrocytes, some hyaline and granular casts. Serum creatinine: 0.15 mmol/l. Neither the hypertension nor the proteinuria decreased after the pregnancy and she was treated with hydrochlorothiazide and methyldopa, which gave satisfactory control of the hypertension.

In 1970, the patient being 27 years old, a renal biopsy was performed. It showed changes corresponding to chronic glomerulonephritis (Fig. 2). Clinical findings at the same time were as follows: BP 130–160/90–120 mmHg. Ophthalmoscopy nothing abnormal. Audiogram nothing abnormal. X-ray of the thorax and [illegible] urography normal. ESR. 30 mm/h. Serum creatinine: 0.13–0.17 mmol/l. Creatinine clearance: 66 and 77 ml/min in determinations. AST and ASH slightly increased. Throat swab: no growth of B-haemolytic streptococci. Immunoelectrophoresis: slightly decreased serum albumin and high [illegible]-globulin. Urinary protein excretion: [illegible]6–7.9 g/24 h with poor selectivity. Examination of serum and urine for amino acids including proline and hydroxyproline normal. Urinary sediment: less than 4 leucocytes and less than 3 erythrocytes per visual field, few hyaline and granular casts.

In 1972 another renal biopsy was made, which showed marked progression of the glomerular changes with hyalinization of more than [illegible]3 of the glomeruli (Fig. 3). At the same time a slight decrease of the renal function taken place, while the degree of proteinuria and urinary sediment were unchanged.

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[illegible]-year-old woman who died in 19[illegible] from uraemia. [illegible] the age of seven she had been known to have [illegible] up to [illegible]. When 22 years old she was [illegible] hospital because of fatigue and headaches. She [illegible] her BP was elevated: 230/140 mmHg. [illegible] 10 mmol/l. Proteinuria [illegible]. Urinary sediment [illegible] leucocytes, no erythrocytes or casts. Urine [illegible]-positive cocci. The patient wished to be [illegible] two days later and received no treatment. [illegible] readmitted at the age of 23 with uraemia, [illegible] headache. The results of the examinations [illegible] were: BP 230/130. Ophthalmoscopy: fundus III–IV. Serum urea: 18 mmol/l. Proteinuria:

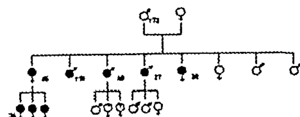


Fig 1 Pedigree of the family The filled-in symbols show family members with renal disease, the numerals the age of death. P = case 1 (propositus), † 24 = case ., unmarked = case 3 † 45 = case 4 † 51 = case 5

12‰. Urinary sediment: normal. She was treated with Serpasil® and Apresolin® without effect, then with Mevasin® which was withdrawn because of dizziness. She was discharged after a .-month stay in hospital, in fair well-being and without prescription of any drugs.

Six months later she was hospitalized for the third time with symptoms as earlier mentioned, as well as visual disturbances and signs of haemorrhagic diathesis. BP 40/140. Serum urea 48 mmol/l. The renal failure progressed quickly and she died two week after admission. At autopsy the organs showed marked signs of uraemia with pleural effusion, pericarditis and ascites. The kidneys were small, the surface finely granulated, and the renal cortex was narrowed but without focal changes. The microscopy showed picture corresponding to chronic glomerulonephritis.

Case 3

A 35-year-old woman who was debile (IQ 77) and had been schooled in a remedial class At the age of 14-15 had several epileptic fits. After continuous treatment Fenemal she has been free of fits. When 20 years had a few attack of sore throat, and in connec- with these a proteinuria of 0.2-0.3‰ was found



Fig 2 Renal biopsy from patient 1 showing glomerulus with an increase of the mesangial space slight hypercellularity and a synechia between the glomerular tuft and the capsule (←). (Placed at disposal by Dr C. Brun)



Fig 3 Renal biopsy from patient 1 ... after the first biopsy showing marked aggravation with mesangial thickening and several hyaline thrombi. (Placed at disposal by Dr C. Brun.)

She was then hospitalized. Nothing remarkable was found at the clinical examination. BP 130/80. Apart from the proteinuria the urine was normal.

In the same year she was examined for her epilepsy Neurological examination showed nothing abnormal. EEG moderately abnormal with diffuse occipital and temporal dysrhythmia, paroxysms and many spikes. A proteinuria of 1-1.5‰ was found again. Eye and ear examination: normal. Renal biopsy was not performed. The patient has not been examined since then, but she is still reported to have asymptomatic proteinuria.

Case 4

A woman, who died 45 years old in 1956 from uraemia. For many years the patient had had proteinuria and hypertension. In addition she had often headaches, nausea and vomiting during the last few years of her life. She had never been extensively examined. In 1956 she was sent to hospital in coma. Laboratory findings: BP 250/130 Hb. 77% Serum urea 26 mmol/l Urine + protein Urinary sediment nothing abnormal.

The patient died on the same day and autopsy revealed haemorrhage with central necrosis in the right cerebellar hemisphere The brain was oedematous The heart was moderately enlarged, weight 400 g with left ventricle wall of 1 mm. The kidneys were strongly diminished, 8 4 2 cm. The surface finely granulated with narrowed renal cortex. There were no focal changes. The other organs showed nothing specific.

Microscopy of the kidneys most of the glomeruli were quite or partly hyalinized, others showed coalescence between the blades of the Bowman's capsules and others thickening of the latter Locally there were proliferating endothelial cells and a few crescent formations. The tubuli and the collecting tubes showed contents of an ample amount of mostly hyaline casts but the epithelium did not reveal any clearcut changes. Interstitially considerable fibrosis was seen and slight lymphocytic infiltration. The vessels were strongly thickened, hyperplastic,

of the hypertension type. Diagnosis: glomerulonephritis, stage III.

Case 5

A 51 year-old man who died in 1961 from uremia. Silent proteinuria was found five years previously. Furthermore there had been frequent attacks of catarrh and sinusitis. In Jan. 1961 he had an episode f macroscopic haematuria, treated with Furadantin® I March there was another attack of haematuria, this time with tenderness over the right kidney and he was once more treated successfully with Furadantin. He now became increasingly tired and was often bothered by headache and blurred vision. Therefore he was hospitalized in April 1961

On admission he was found to be chronically ill and sallowish. BP 230/130. No oedema. Ophthalmoscopy revealed fundus hypertonicus III. Otological examination showed normal hearing of both sides, but subacute ethmoidal sinusitis was found. This was treated with ephedrine drops and Proetz suction. Laboratory findings Hb 49%, ESR: 115 Serum creatinine: 1 1 mmol/L Urine: proteinuria of 2 5‰, 24 hours excretion: 1 6–5.2 g. No glucosuria. The urinary sediment showed at one examination many erythrocytes, on two other occasions no erythrocytes. Many leucocytes were found, but no casts Urine culture was negative.

The condition of the patient gradually declined and he died 1 / months late Autopsy showed an enlarged heart th fresh thrombus in the right coronary artery The lungs were oedematous with pneumonial changes in the right superior lobe. There was an exudate of 200 ml in the left pleura. The kidneys were diminished, 3 4 9 cm. The surface was finely granulated and the cortex strongly narrowed. The papillae were normal and there were no focal changes.

Renal microscopy revealed sparse sclerosed glomeruli Other glomeruli showed necroses, partly focal, partly diffuse, as well as complicating leucocyte infiltration. Some had severe changes of the vessels, with local hyalinization. In some glomeruli slight cell proliferation was seen. The appearance of the tubuli was variable, some were atrophic, while number were enlarged, and in some tubuli precipitation could be seen, occasionally th leucocytes. The amount of connective tissue as somewhat increased The arterioles had thick alls with some signs f obliterating endarteritis, and there was slight hypertrophy of the media. Microscopic diagnosis: malignant nephrosclerosis

Other relatives

Information about these has been obtained from the propositus and maternal aunt, ho is in good health Two maternal uncles died from renal diseases t 37 and 40 years of age A maternal aunt, who died of pneumonia at 28 years of age, suffered from chronic renal disease The grandmother who died from throat cancer 57 years of age, and the grandfather who died from liver cancer at 72, did not suffer from any renal disease as far as is known I the family there is no case of early deafness, eye abnormality or congenital deformatio It has not been possible to contact the remaining members of the family

DISCUSSION

In this family there is a massive accumulation of renal disease. The typical picture has been an insidious development of proteinuria, hypertension and progressing renal failure sometimes with a mild nephrotic syndrome. Ha maturia has been proved only in one of the five persons examined. Eye abnormalities or hearing defects ha e not been found. Microscopically the disease has cor responded to chronic glomerulonephritis and foam cells have not been found. It has attacked men as well as women and the inheritance is apparently dominant.

In the past a considerable number of hereditary renal diseases have been described, and some good reviews have been published in this field (13 14 16). The disease described most often is Alport s syndrome, first described in 1927 (2), later by many others (4 5 9). It is characterized microscopically as glomerulonephritis with foam cells in the interstitium of the kidneys; clinically there are hearing defects, now and then eye defects, usually haematuria but rarely hypertension. A nephrotic syndrome may appear but is rare. Women are mildly affected. Thus there are several differences between the cases in our family and the typical picture of Alport s syndrome

Russel and Smith (17) have described a disease named hereditary haematuria. This disease is ery similar to Alport's syndrome, and the conditions might be identical, although deafness is not reported. Other very similar conditions are de scribed by Goldmann and Haberfelde (8) and by Rome et al. (15).

In some hereditary renal diseases there exists biochemical abnormality for instance hyper prolinaemia and hyperhydroxyprolinaemia (18) or aminoaciduria (7 10). We have examined th propositu for this, and ha e found a normal amino acid level in blood and urine.

In 1961 McCluskey (11) described the so-called nail-pat lla syndrome. In a family with ccumula tion of a disease resembling glomerulonephritis there were also found many cases of nail de formations, patella deformations, and in some cases iliac horns" Hypertension was rare in this family In our family there were n such deformations, and hypertension was a main fac tor I 1969 Albert et al. (1) described a family with a renal disease characterized by the foll

ing findings: women were heavily attacked, with nephrotic syndrome from early chidhood there was no perceptive deafness in the family but un like our family the condition was dominated by haematuria and inevitably led to early death. A somewhat similar case was reported by Moseley and Polak (12). Hereditary renal diseases such as Fabry's disease (6) infantile nephrosis (19) nephritis with myopathy (3) and the hereditary cystic renal diseases are also excluded by the present examinations.

The renal disease in the presented family does not quite resemble any of the earlier published cases of hereditary renal diseases, and thus it contributes to the varied group of hereditary renal diseases.

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EDITORIAL

RAISED ARTERIAL BLOOD PRESSURE—TIME FOR INTERVENTION?

Arterial hypertension is a circulatory disorder that has attracted interest from morbid anatomists, physiologists, pharmacologists and clinicians throughout this century. The attraction of this syndrome to so many specialities is to a large extent due to the interesting illustration it can provide of the regulation of the circulation in man and of different ways of interfering with this regulation. Arterial hypertension as a consequence of various tumors of the adrenal glands has demonstrated the interrelationship between the adrenal medulla and its hormones, of the adrenal cortex and its hormones on the one hand and such variables of the circulatory system as the state of the resistance vessels, the magnitude of blood volume or cardiac output and the height of the arterial BP on the other. Similarly the demonstration—first in animals and later in man—that interference with the renal blood flow may cause arterial hypertension has opened up a large experimental and clinical field for investigation, though the exact mechanism for this relationship is still unknown.

Nobody has argued against calling renal or adrenal hypertension diseases, at the same time as it is clear that such diseases are infrequently seen in the population. The large bulk of clinical cases of arterial hypertension, where no obvious cause for the increased BP can be found, has on the other hand been under thorough scrutiny whether they really represent a disease syndrome—called essential hypertension—or constitute merely the right-hand part of the normal distribution of BP—conveniently called raised arterial pressure of unknown cause. Nobody has questioned that secondary arterial disease can be the consequence of such raised arterial pressure or that the late clinical manifestations of this arterial disease—cerebrovascular, coronary or renovascular—bear some relationship to the height of the arterial pressure, as measured once in a physician's office (the "casual blood pressure"), but also to a series of other variables. The continuous increase in incidence of such late manifestations of arterial disease with the height of the casual BP has precluded any definition of when "arterial hypertension" is present.

Sir George Pickering (6) has repeatedly challenged every medical audience he has addressed in the last 25 years to provide evidence for a fixed dividing line between normotension and hypertension. This challenge has never been accepted because there is no such evidence. The great variability of the arterial BP, regardless of its mean height over the day, has been the object of study since the mid-forties, but the practical consequences of this variability have not been appreciated except in a few laboratories. In view of the many factors entering into the arterial BP at a certain moment it is obvious—as Sir George so eloquently has pointed out—that it is impossible to define values as normal and possible to distinguish from pathologically elevated BP.

Recent studies in larger populations have instead focused on the question, at what height of the BP should the physician intervene? Similarly the necessity for repeated measurements before any decision to intervene is still not sufficiently realized in most cases.

The most recent surge of interest in arterial hypertension is due to increasing possibilities of treatment of the hypertensive state as an aid to prevent vascular disease and vascular catastrophes. Coupled with this knowledge is the awareness that a large part of the male population—both urban and rural—has raised arterial pressure to a degree where it ought to be treated. In most communities it has also been found that many individuals with high BP either do not know that their BP is raised or, though knowing it, have not been sufficiently forcefully advised treatment and thus pass through an asymptomatic but dangerous phase of the disorder untreated.

Through WHO, the Intersociety Commission for Heart Disease Resources, the British Medical Research Council, the Australian Heart Foundation, the Swedish Board of Health and Welfare and probably many other agencies more effort and resources are now being directed to the important questions: how large a problem does raised BP really constitute in the population, and what can and should be done in the short and longer perspective?

Several studies now under way will throw more light on these questions, but it can already be stated that the treatment of raised BP in the population is not confined to the rather simple procedure of screening the population for raised BP and referring everybody with a casual BP above a predetermined level for treatment. The seemingly simple task of defining such a screening pressure is soon confounded by technical questions as to how and when and how often to measure the pressure. The referral for treatment is also not so simple as, except in the most fortunate communities, too few physicians are available who know how to administer treatment in a way that will meet the approval both of the patient, his family and the medical community.

An important and usually not sufficiently appreciated problem in this context is the asymptomatic individual found in a population survey with moderately raised BP. Is his high BP only a normal reaction to the strain of being part of a medical survey—a consequence of what Pickering calls a defence reflex? Or is it an early sign of hypertensive vascular disease. Only time and repeated observations will tell. It is, however, obvious that in view of the risk of late complications everyone found to have a raised BP once must be followed up with repeated measurements ever after whether he is selected for active treatment or not.

The size—and possible outcome—of this problem can be illustrated from the multifactorial preventive trial under development in Gothenburg. Of 238 5[illegible]-year-old men, who

at the first screening observation had an arterial BP of 160–175 systolic or 95–115 diastolic, and who were not advised treatment at the time, tw years later 13 had developed symptoms attributable to an increase of the BP and were put on active treatment. Of the remaining 265 men 20, though asymptomatic, had experienced an increase in BP —to values above 175 systolic or 115 diastolic—and 97 had regressed to normal pressure, i.e. below 160/95. The original 278 individuals who had been defined as exhibiting border line hypertension comprised roughly one-third of all screened men born in the same year 1919 The importance of close surveillance *ithout treatment* of such group of men is borne out by the large proportion—more than one-third—who, without intervention, later exhibited normal BP values. The importance of being prepared to institute treatment i such group as well is similarly show by the substantial umber who with or without symptoms exhibited raised BP with the implications this has for the vascular system. Totally this means that a large part of the middle-aged male population must have access to expert advice regarding BP and hypertensive disease in order to ensure that the dire consequences of raised BP as well as the equally negativ effects of overtreatment are kept under control.

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BOOK REVIEWS

Infectious diseases. Edited by P D Hoeprich. 1200 pp., £ 18.70. Harper & Row London, England 1972.

Infectious diseases remain leading causes of morbidity and mortality throughout the world primarily because known measures of control and therapy are not applied. In technically advanced countries, the pestilences of antiquity no longer occur Yet, even in these areas infectious diseases remain important contributors to illness and death.

This is the beginning of the Editor's preface of the imposing volume Infectious diseases with nearly 100 contributors. The volume has the subtitle: A guide to the understanding and management of infectious diseases. Hoeprich continues his preface: Among clinical speciality areas, that of infectious diseases is remarkable in being non-systemic in orientation. The specialist in infectious diseases must be prepared to deal with involvement in any organ, system, or region of the body In this requirement, the specialty of infectious diseases becomes the last disciplinary domain of general medicine. It is to subserve these broad requirements that this volume was conceived. The aim is synthesis of information rather than encyclopedic assemblage of ideas and data. Students of medicine are the intended audience—primarily the predoctoral and newly fledged physician-in-training, but hopefully also the postresident physician-in-practise.

That is a pretentious and difficult plan. Has it been realized? Nearly all sorts of human infections in industrial as well as in developing countries are dealt with in this book —in the last chapter even diseases of possibly infectious origin, e.g. sarcoidosis and Whipple disease. The only infectious disease I miss is Yersiniosis enterocolitica, a rather important clinical entity in Sweden.

Hoeprich has chosen several of the very best authorities as co-workers, most of them Americans, some English. Generally they have composed very clear and penetrating surveys with a uniform pattern of discussion in all the chapters At the end of each chapter is a short list of references. Only in a few chapters have the authors perhaps been interested a little too much in the theoretical microbiological and patho-physiological aspects, at the expense of practical clinical aspects. Hoeprich has arranged the specific infectious diseases according to the system involved, an approach consistent with the facts of clinical life. This makes it easy for the reader to find a complete discussion of, e.g., bacterial pneumonias or urinary tract infections or wound infections. On the other hand, a complete picture of Coxsackie virus infections, for example, requires consultation of several chapters of the book.

The Editor has managed to keep his co-workers within bounds. Despite the large number of contributors there is surprisingly little overlapping and the discussions of the various topics are of largely equal length. The chapters on streptococcoses and on streptococcal skin infections and glomerulonephritis are good, though possibly somewhat long. The chapters on infectious hepatitis and serum hepatitis are, however altogether too brief. Many new facts of these important infections are missing. Also cytomegalic inclusion virus infection is dealt with somewhat briefly

Hoeprich himself has written the chapters on infective endocarditis, bacterial meningitis, and bacterial pneumonias —excellent writing by an experienced clinician and active scientist.

Some minor remarks: Regarding the cause of bacterial pneumonias Hoeprich believes that the pneumococci still account for 90% of the cases. This figure seems high for Scandinavia. The very difficult differentiation in clinical work between pulmonary embolism and pneumonia should perhaps have been given more space.

Several other chapters are also very well written, especially those on gastroenterocolitis and salmonellosis by Hook and Johnson.

The section on wound infection as well as the chapter on staphylococcal skin diseases make good reading also for surgeons—though the daily bathing of infants with hexachlorophene-containing soap is already obsolete.

The first three sections, 200 pages, of the large volume are devoted to general problems of infectious diseases, most of them written by Hoeprich himself. The first section, on epidemiology host-parasite relationship and on factors bearing on the development of infectious diseases, is very interesting. To the Swedish reader section II, devoted to laboratory examinations, seems unnecessary in a book like this. It is a good refresher but not a substitute for special literature. In section III, on control of infectious diseases, Hoeprich has written a fine survey of antimicrobial therapy including side-effects. The review is both lucid and concise. In the following chapter on prophylaxis a Scandinavian reader observes Hoeprich's relatively narrow range of indications for BCG vaccination and his interest in "chemoprophylaxis" of persons exposed to tuberculosis—a view different from that prevailing in Scandinavia.

To edit a book like this, a book intended to impart understanding as well as knowledge of the practical management of the entire panorama of human infectious diseases, a panorama which appears very different from different aspects of the globe, is a difficult task. Hoeprich and his co-workers have been largely successful. This is a fine volume, filled with interesting knowledge, and, to the specialist of infectious diseases, necessary knowledge. The Swedish students of medicine will not have time for a book like this, but it is nearly a "must" in hospital libraries in Sweden. Hospital doctors of all specialities should have easy access to the book, in which they can readily find accurate and concise knowledge in this field, so important to all of us.

Sven Belfrage

Acta

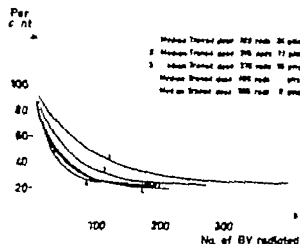


Fig 4 Development and degree of lymphopenia during ECIB in relation to different TD

after 28 No. BV rad. at 400 rads in TD and after 18 No. BV rad. at 500 rads in TD

3) *Varying frequency of the ECIB sessions.* The median frequency of the ECIB sessions was every 1 8th day both in group I and group II. Comparing the development and degree of the lymphopenia during ECIB for 35 patients treated with a frequency less than median (every day to every 1 7th day) with that of 36 patients treated at greater intervals (every 1 8th to every 4th day) no difference could be demonstrated in these parameters. Four patients were treated in very long sessions (15.5–23 7 hours per day) every day to every 2nd day. Six patients were treated with ...odialysis only twice weekly. Ceteris paribus ...development and degree of lymphopenia during ECIB in these two groups did not differ from the findings for the rest of the patients.

Duration of lymphopenia after the end of ECIB. The lymphocyte concentration was followed in 19 patients who were not transplanted in the period 5–18 months after cessation of ECIB. Group A (9 patients) had received a low transit dose of 85–100 rads and 1 –4 6 No. BV rad./hour during ECIB. Group B (10 patients) had received higher transit doses (195–505 rads) and 0 8–1 6 No. BV rad./hour. The median percental lymphocyte concentration after the end of ECIB is shown in Fig. 5. In group A all 9 patients were followed during 5 months, 6 of them during 9 and 3 during 14 months. In group B all 10 patients were followed during 5 months, 8 of them during 9, 6 during 1... and 3 during 16–18 months.

In group A there appears to be an increase in the median lymphocyte concentration from ..8% to 40% of pretreatment value during the first month after the end of ECIB but thereafter no further increase was observed. The concentration remains stable around 40% in the next 10 months. The increase in lymphocyte concentration was not significantly correlated to the interval 0–5 months after the end of ECIB ($r = 0.110$, $p = 0...$). In group B a slow but significant increase in the median lymphocyte counts from 23% to 35% of pretreatment value was observed within the first 5 months ($r = 0.84$, $p < 0.01$); thereafter the concentration levelled off at 35–40% of pre ECIB value from the 5th to the 10th month after cessation of ECIB. Comparing the mean percental lymphocyte concentration within the first 5 months after cessation of ECIB in groups A and B no significant difference could be observed ($p > 0.1$). In the high TD group B the mean percental lymphocyte concentration within the first 5 months after cessation of ECIB was compared in 4 patients who received a MCED below 50 000 rads (33 170–44 430) and 6 who received a MCED above 50 000 rads (55 540–85 150). The increase in lymphocyte concentration in these two groups was identical ($p > 0.1$).

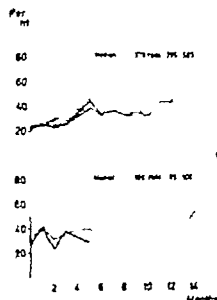


Fig 5 The median monthly lymphocyte concentration in % of pre ECIB level in 19 patients followed during 5–18 months after cessation of ECIB. Upper part group A (— 9 patients followed during ... months, 6 during 9 ... 3 during 14). Lower part group B (— 10 patients followed during ... months during 9 — 6 during 1... 3 during 14)

DISCUSSION

The basic experimental research on ECIB was performed by Cronkite and coworkers (1 2, 3 12). They have shown the efficiency of ECIB in producing lymphopenia in the peripheral blood and the lymphoid tissue (1 12). Calves were treated with ECIB continuously for 40 hours with a TD of 900 rads. After 10 hours of ECIB the lymphocyte concentration in the blood was reduced to 20% of the pre-ECIB level during the following 10–40 hours of ECIB no further reduction was seen. In the lymphoid tissue the cell depletion was quantitated by means of planimetric measurements of the densely populated areas (1). All of the lymphoreticular tissues showed a two-component depletion curve an initial rapid decrease in lymphocyte concentration during the first 10 hours of ECIB to approximately 75% of pre-ECIB level, followed by a slower decrease during the following 10–40 hours of ECIB. Comparing the depletion curves for blood and lymphoid tissues it appears that the depletion of the tissue lags behind depletion of lymphocytes from the peripheral blood. Although it seems likely that both the T and the B lymphocytes recirculate between the blood and the lymphoid tissue, the above mentioned finding might be explained by the fact that the traffic time" in the lymph nodes for the B lymphocytes seems to be longer (24–28 hours) than for the T lymphocytes (14–18 hours) (5 6, 8, 9).

Cronkite and coworkers found a direct relationship between the TD and the degree of lymphopenia for TDs between 15 and 500 rads. Using TDs between 500 and 900 rads no significant difference in the degree of lymphopenia production was found, which might indicate that the "killing dose" for lymphocytes in calves is about 500 rads. Furthermore it appeared that repetitive short sessions of ECIB were more efficient in producing lymphopenia than continuous ECIB.

The return of blood lymphocytes to the average pre ECIB level in cal es was dependent upon the extent f ECIB-induced lymphopenia. When the lymphocyte concentration was depressed to only 40 of pre-ECIB lev l the previous level was rea hed after 1 month, whereas a reduction to 20 results in a stable lymphopenia within 10 mo ths or more after cessation of ECIB.

T results presented in this study are in accordance with the experimental findings. In patients with chronic uremia, undergoing ECIB, the lymphocyte concentration in the blood was reduced in the same characteristic way after an initial rapid decrease to 25% of pre ECIB level a new "base level" was achieved. The development and degree of lymphopenia were also closely related to the TD although higher TDs than 500 rads were not examined in this study The size of the exchangeable pool of small lymphocytes in humans is assumed to be 20–40 times the size of the lymphocyte pool in the blood (4 11). In the present study the exchangeable" pool was halved after about 18 No. BV rad. when a TD of 500 rads was used. This indicates that the "killing dose" for human lymphocytes, similar to that of calf lymphocytes, is about 500 rads, a lethal radiation dose which is in good agreement with chromosome studies (4). Differences neither in the blood flow rate in the patient s shunt or fistula nor in the frequency of the ECIB sessions, within the limits of the present study seem to have any demonstrable influence upon the development of lymphopenia.

After cessation of ECIB a more rapid increase in the lymphocyte concentration was also seen in the group of patients who showed a less pronounced lymphopenia during ECIB (group A). The steeper increase was, however only observed within the first few months after the end of ECIB. When a pre ECIB level of 35–40% was reached, no further increase could be demonstrated during the following 5–10 months, either in the low TD (group A) or in the high TD patients (group B). Preliminary results, presented in a preceding paper (14), showed that the lymphocytes increased to 50% of pre ECIB level 3 months after cessation of ECIB in patients who had received a low TD of 100 rads. In the present study more patients are included and nonparametric statistical evaluation of the results has been performed. This might explain the differences.

Human peripheral blood lymphocytes are very heterogenous with regard to life span, origin and function. On the assumption that the production of lymphocytes in the primary lymphoid tissue (bone marrow and thymus) is constant and not dependent on the concentration in the peripheral blood, the present results seem to indicate the existence of at least three populations of lympho-

cytes with regard to life span. Approximately 60% of the peripheral lymphocytes are very long-lived (years) and are easily destroyed during ECIB. The remaining 40% might be composed of a population of cells with a shorter life span (months) which is destroyed, too, during ECIB but reproduced during the first 5 months after cessation of ECIB and a population of lymphocytes with a very short survival time (days), in which the rate of destruction during ECIB equilibrates with the rate of production. With regard to function, the relative proportion of T lymphocytes in the reduced lymphocyte population seems to be unchanged (15). The per centile number of B lymphocytes, determined by means of immunofluorescence technique is under investigation (7, 16). In a recent study lymphopenia was induced by irradiation for mammary carcinoma (13). A significant decrease in the proportion of T lymphocytes and a relative increase in B lymphocytes was found in these patients, but this finding might be explained by the fact that part of the thymus gland was involved in the irradiation procedures.

Based upon the present findings the most efficient schedule of ECIB in producing lymphopenia in clinical work cannot be stated. Meanwhile it may be relevant to use a TD of about 500 rads, which seems to destroy approximately 100% of the lymphocytes during a single pas- of the irradiator. The No. BV rad. should [illegible]uch that the "base level" of the produced [illegible]phopenia has been reached, i.e. about 100 [illegible]ith a TD of 500 rads. This gives an MCED of 50 000 rads. Histological examination of the lymph nodes, which might elucidate whether a lower MCED which apparently gives a stable but less pronounced lymphopenia in the blood, can sufficiently deplete the lymphoid tissues, has not been performed in these patients.

Our present ECIB schedule before transplantation is as follows: TD 500 rads, No. BV rad. 100 (assuming a BV of 5 l), MCED 50 000 rads. With a blood flow rate from shunt or fistula between 100 and 200 ml/min the average duration of one therapy session is 50–100 hours.

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

VII. *Longitudinal Course during Terminal Renal Failure and Regular Hemodialysis*

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Abstract. Serial determinations of the sensory and motor nerve conduction, and of the vibratory perception threshold (VPT), and serial clinical neurological examinations have been performed in 16 patients during progressive renal failure and/or regular hemodialysis. During progressive renal failure gradual and almost equal slowing of the nerve conduction was observed in lower as well as in upper extremities, although clinical neuropathy was prevalent in the lower extremities. In contrast to the gradual slowing of the nerve conduction, clinical findings usually developed abruptly, the first indication being a sudden rise in VPT. During regular hemodialysis there was no further slowing of the nerve conduction, nor was there any significant improvement. Despite this fact a marked decrease in VPT occurred within the first months, followed by a somewhat slower remission of other clinical findings. The present study thus confirms and adds further evidence to an existing dissociation between clinical findings and nerve conduction data, demonstrated in a previous study based on single observations in a larger material of patients with varying degree of renal failure. With the aim of preventing clinical neuropathy VPT is advocated as the most valuable and simple method among the neurological variables studied for the selection of the optimal time for institution of regular hemodialytic treatment.

In a previous report (17) based on single observations in 56 patients with varying degree of chronic renal failure it was demonstrated that there was no relation between the peripheral nerve conduction velocity and the prevalence, distribution and severity of clinical neuropathy. In the present report this problem is evaluated in further detail from a longitudinal study in a series of patients followed during terminal renal failure. Furthermore after institution of regular hemodialysis the pattern of changes in the clinical picture, the vibratory perception threshold (VPT) and the sensory and motor nerve conduction velocity were studied during partial control of uremia. The VPT is advocated as a simple and relevant indicator of the induction and remission of clinical neuropathy, suitable in determining the optimal time for commencement of hemodialytic treatment and for the control of its effect.

MATERIAL AND METHODS

Sixteen patients, 4 females and 12 males, 21–48 years of age (average 33), were examined prior to and/or during the early period of our regular hemodialysis program in 1965–69 (Table I). The histological diagnosis (kidney biopsy or nephrectomy) of the primary kidney disease was chronic glomerulonephritis (7 patients), chronic pyelonephritis (7 patients), polycystic kidneys (1 patient), and malignant nephrosclerosis (1 patient). The residual kidney function (24-hour endogenous creatinine clearance, C_{cr}) before dialysis, transplantation or death was on average ...4 ml/min (Table I). The material was divided into two groups: A. Thirteen patients, followed during the terminal part of chronic renal failure for 1–14 months (mean 5). One of them was transplanted without preceding hemodialysis, and one died before dialysis became possible. B. Fourteen patients, followed during regular hemodialysis. These comprised the 11 remaining patients of group A and another three patients who were only examined once during terminal renal failure immediately prior to dialysis. The observation period was 1 $/_{2}$–13 months. Six of the 14 patients were transplanted after 1 $/_{2}$–9 months of regular hemodialysis, two died after 3 months, while six were still on the dialysis program after 1 year. Thus, among the 16 patients, 11 were followed during terminal renal failure as well as during regular hemodialysis.

Hemodialysis procedure. All patients were dialyzed 8–10 hours twice weekly with a modified ...-layer Kiil kidney. This was a single-pass, non-recirculating system using Cuprophane PT 150 (Bemberg) or viscose membranes (American Viscose Company, Frederiksberg) with

Table I *Patient data*

GN glomerulonephritis, PN = pyelonephritis, PCK = polycystic kidneys, MNS malignant nephrosclerosis, HD hemodialysis, RAT = renal allotransplantation

Pat. no.	Sex	Age (y.)	Diagnosis	C_{cr} (ml/min)	Months on hemo-dialysis	Clinical neuropathy 1st exam.	Pre dial.	Post dial.	Course
6	♂	47	GN	6.3	12→				HD
7	♂	40	PCK	3.8	3	0			HD→RAT
32	♂	79	GN	1.0	12→		( )	0	HD
35	♂	3	GN	3.7	12→			0	HD
41	♂	48	PN	1.0	12→	+	( )		HD
47	♀	27	GN	1.0	9	0			HD→RAT
48		29	PN	2.8	1→			0	HD
61	♂	21	GN	1.0	3	0		( )	HD→†
63	♂	41	GN	3.8	12→			0	HD
66	♀	31	PN	4.2	2	0	0	0	HD→RAT
92	♂	34	PN	2.2	–	0	+ ( )		†
109	♀	42	PN	1.2	—				RAT
124	♂	33	MNS	1.4	3	( )			HD †
126	♂	25	PN	1.0	1	0	( )	( )	HD→RAT
131	♂	32	GN	3.0	3	+			HD→RAT
132	♂	35	PN	1.0	2				HD RAT

* 0 absent, mild, moderate, severe.

of 1.4 m² and a urea clearance of bout 80–100 ml min (4). Between dialyses the patient were given low-sodium, low-protein diet supplemented ith vitamins. Serum urea ranged from 0.3 to 1.5 g l post predialysis. The hydration was controlled by w ter restriction and dialytic ultrafiltration and the serum lectrolyte concentrations showed only small variations within the normal range. If nec ry a potassium-binding resin (Resonium®) was en to control the serum potassium lev l BP could be trolled by sodium restriction ithout additional antirtensi e drugs, in 4 of the 16 patients, however nly fter bilateral nephrectomy. During long-term dialysis the lean body mass steadily increased. After 1 months treatment was continued on an ambulatory basis and most patients ere ble to resume their normal ork.

Neurological examination. In addition to routine neurological examination the *VPT* (VT) was measured on the pulp of the big toe (PH) and on the medial malleolus (MM) using a Biothesiometer® (Chagrin Falls, Ohio, Mass.) (15).

Nerve conduction studies in the median and common peroneal nerves comprised the distal motor latency (msec) from the rist to the abd poll brevis muscle and from the ankle to the ext dig. brevis muscle and the motor conduction elocity (m sec) bet een the elbow and the wrist and between capitulum fibulae and the ankle. The sensory conduction elocity (m sec) as examined in distal (digit-wrist) and proximal (rist-elbow) segments of the median nerv following supramaximal stimuli to the distal phalanx of digit 1 or 3 (.., 16 20). The extremity was heated prior to and during the examination with radiant lamp and temperatures ere recorded near the nerves with a thermocouple. The mean of proximal and distal measurements wa near 35°C. Conduction locities er not corrected for te pera ures since each patient served as his own control. Normal alues gi en in this paper ere mean values in 34 persons, 20-40 years of age (median nerv (20)), and 4 persons, 20-60 years of age (peroneal nerv (16)).

Intraindividual variation. VPT (PH and MM) as mea ured twice in 19 normal persons and 28 uremic patients ith levated thresholds, the interval ranging from 1 to 11 da . On a logarithmic scale (log₁₀ VT) the intraindividual variation was the same in the rmal and the pathological range. $F = 1.68$ (PH) and 1.06 (MM), $p > 0.10$. For the total group (47), the 95 r nge of intraindi idual variation (= ± times the S D of the mean differenc between 1st and 2nd determination) ±0.2978 log V (PH) and +0.354 log VT (MM). The 95% range of intraindi idual ariation in the nerve conduction velocities appears from T ble II (20).

Statistical analysis. I paired comparison the mean difference

$$\bar{X}_D = \frac{\Sigma D}{N}$$

where D = the difference between tw observations in the same person and N number of person. The standard deviation of the difference

$$s_D = \sqrt{\frac{\Sigma D^2 - (\Sigma D)^2/N}{N-1}}$$

The standard error of $\bar{X}_D$

$$s_{\bar{X}_D} = \frac{s_D}{\sqrt{N}}$$

Table II. *Sensory and motor nerve conduction (m/sec) in terminal renal failure (A) and during regular hemodialysis (B)*

W = wrist, E = elbow CF = capitulum fibulae A = ankle

		Segments				
		Median nerv				Common peroneal nerv
		Dig. 1 W	Dig 3-W	W-E	E W	CF A
Normal mean value (S D.)		54.2 (3.9)	59.6 (3 4)	66.4 (4.5)	62 8 (4 2)	50 0 (3.5)
Intraindividual variation		±2.6	±3.6	±1.8	±3.2	
Pat. no.	Interval (mo.)					
A						
7	6	52–49	58–56	59–51	57–52	41–41
41	3	44–41	47–48	49–47	50–48	30
47	3	48–42	52–48	57–54	51–42	33–(0)[b]
61	10	49–43	58–50	68–53	64–53	49–29
66	14	50–45	55–52	57–57	60–56	45–44
92	2	43–38	48–42	52–47	48–41	32 27
109	1	44–38	49–	55–47	52–48	44–34
126	5	49–39	50–44	53–49	50–46	40–28
132	2	48–44	51–49	58–58	50–47	35–29
Mean difference (±S.E.)		−5.3±0.71	−3.8±1.01	−5.0±1.39	−5.4±0 99	−7 7±2.62
p		<0 001	<0 01	<0.02	<0 001	<0.05
B						
7	3	49–48	56–55	51 52	52 55	41 31
35	13	39–46	43–50	52–60	53–55	41–43
41	8	41–47	48–49	47–49	48–46	30–32
47	9	42 36	48–37	54–32	42–19	(0)–(0)[b]
48	6	51–55	55–59	69–59	55–55	42–40
63	6	41–40	46–47	57–54	57 52	43–41
126	1	39–41	44–43	49–51	46–45	28–22
131	2	46–46	51 53	59–58	52–52	40–35
132	2	44–45	49–48	58–53	47–47	29–33
Mean difference (±S E.)		0 9±1.38	+0.1±1 65	−3.1±2.90	−2.3±2.63	−2.1±1 67
p		>0.5	>0.9	>0 3	>0 3	>0.2

95 % range. [b] Ext. dig. brevis muscle inexcitable.

Whether $\bar{X}_D$ differs significantly from zero was tested by the Student's t-test, where

$$\frac{\bar{X} - 0}{s_{\bar{X}}} \quad = N - 1 \text{ degrees of freedom.}$$

RESULTS

Nerve conduction

A. *Terminal renal failure* (Table II A). The nerve conduction was followed for 1–14 months (mean 5 1) in 9 of the 13 patients. In the *median nerve* the mot conduction elocity in the elbow–wrist segment showed an a erage decrease of -5.4 ± 0.99 m sec (p 0 001), whereas the distal motor latency was unchanged ($\bar{X}$ = 0.2 msec, $p > 0.10$). A sign fica t lowing of the conduction velocity was lso prese t in distal and proximal segments of sensory fibers ($p < 0.001$ and < 0.02 respectively). In the *common peroneal nerve* the motor conduction velocity became slowed by -7.7 ± 2.62 m/sec ($p < 0.05$). There was also a significant increase in the distal motor latency ($\bar{X}_D$ = 1.8 ± 0.65 msec, $p < 0.05$), but this was at least partly due to an a erage increase of the inter electrode distance of 11 mm between the ankle and the ext. dig. brevis muscle. In patient 47 the motor conduction was severely reduced at the first xamination (33 m/sec), but immeasurable 3 months later as the ext. dig brevis muscle had become inexcitable by supramaximal stimuli. This patient was therefore excluded from the statistical analysis

Fifteen of the 16 patients were examined shortly before dialysis, renal transplantation, or

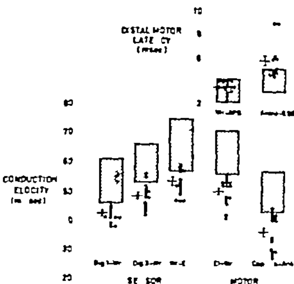


Fig. 1. Sensory and motor conduction velocities (m/sec, left scale), and distal motor latencies (msec, right scale) in end-stage kidney failure. Shaded area = 95% of normal range. (*W* = wrist, *El* = elbow, *APB* = abd. poll. brevis muscle. *Cap. Fib.* = capitulum fibulae, *EDB* = ext. dig. brevis muscle).

death (Fig. 1). The reduction of the mean conduction velocities ($p<0.001$) amounted to about 20% of the normal mean value in all segments of the median nerve, and to 28% in the common peroneal nerve. The distal motor latency in the median nerve never exceeded the upper normal limit and was significantly increased in the common peroneal nerve in half of the patients.

B. Regular hemodialysis (Table II B). The nerve conduction could be reexamined in 9 of the 14 patients during the first year of dialysis (mean observation period 5.6 months), as one patient was soon transplanted, two suddenly died, and one refused reexamination. There was no significant change in the distal motor latencies nor in the sensory or motor conduction velocities during regular hemodialysis ranging from 1 to 13 months. Two patients showed changes in conduction velocity greater than the expected intraindividual variation. Patient 35 obtained almost normal conduction velocities after 13 months of dialysis, while in patient 47 the progressive slowing of sensory and motor nerve conduction in the median nerve, observed during terminal renal failure, continued during 9 months of regular hemodialysis. (The nerve conduction improved considerably shortly after renal transplantation (19).)

In the two groups of patients described, differences in temperature near the nerve between two examinations never exceeded 2.0°C, the mean differences ranging from −0.2°C to −0.5°C. When the conduction velocities were corrected to a standard temperature (correction factor = m/sec/°C (3)), a minor reduction of the differences was obtained. This, however, did not alter the statistical significance of the results.

Vibratory perception

A. Terminal renal failure (Fig.). A significant increase in VPT (PH and/or MM) was recorded in 8 of the 13 patients during terminal renal failure. The average difference was more pronounced in the big toe (0.5848 ± 0.1676 log V^2, $p<0.005$) than on the malleolus (0.3192 ± 0.1771 log V^2, $p<0.05$). Thus, the VPT PH/MM ratio normally <1 became inversed in 11 of the 13 patients. The material, however, showed great variations, as seen in Fig. , not only due to differences in observation time. VPT remained normal in two of eight patients with normal VPT values at the first examination. Three of five patients with pathological VPT values at the first examination showed no further increase, but all three were followed for less than 2 months. The developement of impaired vibratory perception

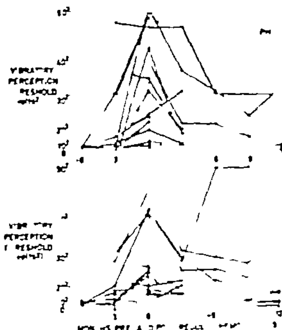


Fig. VPT (V²) before and during regular hemodialysis. The ... increase and decrease in VPT ... both test sites before and during dialysis ... significant.

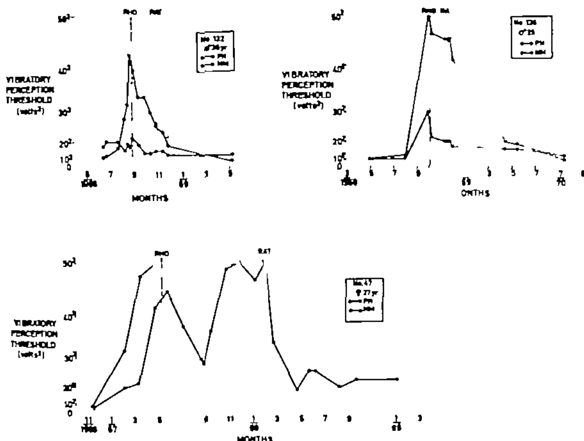


Fig. 3 Individual courses of VPT on PH and MM. *RHD* = start of regular hemodialysis, *RAT* = renal allotransplantation. The three graphs demonstrate the abrupt rise in VPT_{PH} in terminal renal failure. Note also the characteristic inversion of the VPT PH/MM ratio, which returned to normal after renal transplantation in the upper two patients.

Regular hemodialysis was commenced before VPT_{MM} had become significantly impaired (upper right), or early in the rising phase (upper left). In both patients the clinical course was favorable. Remission of clinical neuropathy, most severe in patient 126, was seen during dialysis, and was completed after renal transplantation.

In patient 47 (bottom graph), both VPT_{PH} and VPT_{MM} had been considerably elevated for some time before dialysis was started. The clinical course during dialysis was unfavorable. Clinical neuropathy tended to disappear along with the temporary improvement of VPT_{MM}. The secondary rise in VPT_{MM} after three months, however, announced a severe relapse of clinical neuropathy. VPT_{MM} became almost fully reversed following renal transplantation, while other clinical signs of neuropathy remitted only partly during 2 ½ years of observation. The graph illustrates the practical value of serial determinations of VPT when the vibration sense on the big toe becomes extinct.

was abrupt (Fig. 3). VPT might remain normal for months despite a progressive slowing of the nerve conduction. Then, within weeks, VPT on the big toe suddenly increased almost linearly in most patients later followed by an equally abrupt but usually less pronounced rise of the VPT on the malleolus. This was neither correlated with any sudden change in nor with any critical level of the nerve conduction.

B. *Regular hemodialysis.* VPT(PH) was significantly elevated in 10, and VPT(MM) in 7 of the 14 patients when taken into regular hemodialysis. VPT decreased significantly on both test spots during the first year (-0.3089 ± 0.0831 log V^2, $p < 0.005$ (PH), and -0.2114 ± 0.0938 log V^2, $p < 0.05$ (MM)). The major part of the improvement took place during the first 3 months of treatment (Fig. 2). Recovery however was rarely complete, but continued in some patients during the following months. Two patients showed a course different from the rest of the group. In patient 61, male 21 years old, the rise in VPT

observed in terminal renal failure continued during the 3 months on regular hemodialysis until he suddenly died. This was accompanied by a further progression in clinical neuropathy. In patient 47 (Fig. 3) the vibratory perception remained extinct on the big toe for 9 months of regular hemodialysis, while on the malleolus VPT initially showed a gradual but significant improvement, after 3 ¹ ₂ months followed by a second rise. This announced a relapse and further deterioration of the clinical neurological state.

Normalization of VPT during regular dialysis occurred despite unchanged slowing of the nerve conduction.

Other clinical findings (Table I)

Patients who retained normal VPT at most showed weakened or absent deep reflexes (14), which reappeared during regular dialysis. The clinical course was otherwise uneventful. Reflex disturbances might precede impairment of the vibratory perception, but the rise in VPT on the big toe, observed in eight patients, was always followed by more severe sensory-motor signs of neuropathy, the earliest being paresis of the dorsiflexion of the big toe, which became manifest within the first month after the rise in VPT. Affection of other muscle groups and sensory loss developed later in the well known ascending pattern.

With two exceptions (patients 47 and 61) regular hemodialysis interrupted the progression of clinical neuropathy, and development of neuropathy during dialysis was not seen. The decrease in VPT was accompanied by remission of sensory symptoms (within weeks) and improved muscular strength. The remission of other neurological signs, however, lagged considerably behind. Thus, areflexia and pareses were still demonstrable after several months. The investigation, however, was inconclusive as regards the final degree of recovery during dialysis, as most patients with moderate to severe neuropathy were transplanted within the first year, while those who were subjected to long-term dialysis (> 1 year) were either clinically normal or only mildly affected when taken into the program.

DISCUSSION

The high incidence of impaired nerve function before dialysis, especially of slowed nerve conduction, is consistent with most comparable reports (3, 5, 7, 8, 9, 10, 12, 21). This was also expected from the strong correlation with the kidney function (C_{cr}) previously reported (17). Slowing of the nerve conduction was an early finding, as a rule present before other evidence of neuropathy became apparent. The nerve conduction deteriorated gradually and slowly in all nerve segments in the upper and lower extremities, but it is interesting that the distal motor latency was clearly less frequently affected than the motor conduction velocity in a more proximal segment of motor fibers. In contrast to the slow deterioration of the nerve conduction velocity the increase in VPT was abrupt, and preceded the development of a disabling neuropathy in all patients. Thus, the longitudinal neurological course in individual patients during terminal renal failure confirms the previously demonstrated dissociation between the peripheral nerve conduction and clinical findings (17).

This feature came out even more clearly from the course during regular hemodialysis. According to the criteria given by the Seattle group (10), the dialysis was "adequate" in [illegible] of our 14 patients in that 1) development of neuropathy was prevented, [illegible]) progression arrested and 3) evidence of recovery demonstrated. However, recovery was only seen in the VPT and other clinical findings, being demonstrable as soon as partial control of uremia was established. The nerve conduction, on the other hand, failed to improve during several months of "adequate" dialytic control of uremia (2[illegible]). The fact that slowed nerve conduction rapidly improved following successful renal transplantation, also in patients included in the present study (19), clearly indicates that slowed nerve conduction was actually readily reversible as was VPT and other clinical findings. Within a few weeks, regular hemodialysis resulted in a low average concentration of urea and creatinine reaching a level corresponding to mild chronic renal failure, where the nerve conduction is usually normal. Hence the absence of improvement of the nerve conduction during hemodialysis provides further evidence in favour of the concept (17) that the impulse propagation is impaired by toxic substance( ) of a higher molecular weight and with a considerably lower clearance through the artificial kidney than urea and creatinine (17, [illegible]3). On the other hand the progressive

slowing of the conduction velocity during terminal renal failure *become arrested* after the institution of regular hemodialysis. This indicated that an equilibrium became established between the endogenous production and the clearance of the alleged high molecular toxin(s). Evidence in favor of this concept has also been presented both in in vitro studies (1) and in vivo examinations (24). The equilibrium may become disturbed if the catabolic rate becomes temporarily or permanently accelerated during the hemodialytic treatment. This may account for the relapse and further deterioration in neuropathy seen in patient 47 who experienced a series of severe infections and surgical complications in connection with a two-step bilateral nephrectomy

Most dialysis centers have experienced the immense problems in revalidating patients who have developed severe neuropathy Hence, it is important to select a reliable measure to predict and control the development of clinical neuropathy The value of a *routine clinical examination* (6), even when including a scaled system for grading of the neuropathy (21), is limited by the fact that neurological symptoms are inconstant, and that mild signs may appear long before dialysis becomes indicated by uremic symptoms other than neuropathy When finally more severe signs appear clinical neuropathy often progresses rapidly and the optimal moment for commencing diaysis has passed. During adequate dialysis clinical signs tend to disappear *slowly* and it is often very difficult to assess objectively the effect of dialysis.

Jepsen et al. (10) advocated serial determination of the nerve conduction velocity every three months from an early stage of chronic renal failure. In contrast, referring to a great day-to-day variation, Kominami et al. (11) claimed that the nerve conduction is an unreliable tool when major therapeutic decisions are to be taken" Furthermore, slowed nerve conduction was poorly correlated with clinical neuropathy (17). In our experience, slowed nerve conduction could be expected in half of the patients when the kidney function became reduced to 8–10% of the normal (C_{Cr} 8–10 ml/min/1 73 m²). Th nerve conduction deteriorated *gradually* during terminal renal failure, and it was *not* possible to define any critical value where the appearance of clinical neuropathy was particularly imminent Thus, an interval of three months between determinations is too long, since the breakthrough of severe neuropathy from a clinically normal state may take place within weeks. Finally as shown in this and other studies, clinical remission during adequate dialysis treatment took place in spite of an unchanged slowed nerve conduction. In addition, in our experience nerve conduction determinations were unpleasant to the patients and complicated, necessitating expensive equipment and a highly specialized laboratory staff

The *vibratory perception threshold* may turn out to be the most sensitive indicator of imminent neuropathy When expressed in terms of log V^2 the intraindividual variation was independent of the actual VPT level. In *serial determinations* changes in VPT preceded other clinical evidence of neuropathy both during progression and during remission of neuropathy As long as VPT_{FII} remained normal, in some patients for several years before and during regular dialysis, disabling neuropathy never occurred. The first indication of impaired vibratory perception was an *abrupt* rise in VPT on the big toe. This was always an ominous sign, announcing the development of severe neuropathy which became manifest within the first month. A rapid fall in VPT after onset of regular hemodialysis was accompanied by remission of other clinical signs of neuropathy although more retarded, whereas an uninterrupted rise in VPT after onset or a secondary rise during regular hemodialysis indicated further progression or relapse of neuropathy As shown by Kopple et al. (13) VPT may even reflect the effect of single dialyses. VPT is easily determined with a simple apparatus well suited for repetitive routine examinations.

ACKNOWLEDGEMENT

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

VIII. *Recovery after Renal Transplantation. Clinical Aspects*

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Abstract Clinical neuropathy has been studied before and after renal transplantation in 27 patients who received transplant from close relative. With one exception, excluded from the follow-up study uraemia was eliminated in all patients within the first week. Three patients later died from rejection and two from extrarenal complications. The observation period ranged from 1 to 31 months (average 12). The material was divided into two groups: I. Among 14 patients with normal vibratory perception threshold (VPT) on the big toe before transplantation, four had clinical signs of mild neuropathy which disappeared 1–3 months after transplantation. II. Thirteen patients with elevated VPT had other clinical signs of moderate to severe neuropathy. The remission after transplantation was two-phasic with an early rapid and a late slow phase. A marked reduction in the VPT during the first weeks preceded the disappearance of sensory and motor signs, which took place in distalward direction. Muscular weakness and wasting and reflex disturbances were among the latest signs to disappear. A prognostic evaluation of the neuropathy was possible after about 6 months. Clinical signs which had shown no tendency to regress at that time apparently became permanent sequelae. After 2 / years two patients still had paralysis of distal muscles in the legs and of isolated muscles in the hands. The ankle reflexes and the vibration sense on the big toe were extinct and there was severe hypaesthesia of the feet.

The earliest account of the recovery from uremic neuropathy after a successful renal transplantation (in identical twins) was given by Tyler in 1962 (12). Chaumont et al. (2) and Funck Brentano et al. (4) showed that impaired nerve conduction improved considerably but the recovery of electrological signs was slow dissociate, and incomplete as compared with the clinical picture.

Recovery could be expected even in severely affected patients provided that a normal kidney function was preserved. These conclusions were also drawn by Bolton et al. (1). Dobbelstein et al. (3) observed that the clinical remission had a two-phasic course with an early rapid and a late slow phase.

The present and the following paper (10) report the results of a longitudinal study concerning clinical and electrophysiological aspects of the remission from uremic neuropathy during 2 $/_2$ years after successful renal transplantation. The above mentioned two-phasic course was studied in further detail with particular attention to the early postoperative phase. In keeping with previous observations (9) the vibratory perception threshold (VPT) proved to be the most valuable indicator of the clinical neurological course. The results suggested a certain dissociation between the recovery of sensory and motor disturbances. A prognostic evaluation concerning permanent sequelae appeared possible about 6 months after the transplantation.

MATERIAL AND METHODS

The study covers the period Nov 1967 – July 1970 and comprises 27 patients, 14 females and 13 males, 17–51 years of age (Table I). These include the first eight patients transplanted at Rigshospitalet, Copenhagen, and 19 of 21 consecutive patients transplanted at the Municipal Hospital, Arhus. (Two patients were excluded, as the pre transplantation neurological examination could not be planned in advance.) Twenty-two patients had been on regular hemodialysis program for 1–21 months prior to transplantation, while the preoperative treatment comprised occasional peritoneal dialyses in five.

Renal transplantation. Patients 47 and 100 received

A preliminary report on this and the following study (7) was given at the 19th Congress of Scandinavian Neurologists, Arhus, Denmark 1970

Table I. *Clinical data on 27 patients treated with renal allotransplantation (RAT)*

PCK = polycystic kidneys, GN = glomerulonephritis, PN = pyelonephritis, MNS = malignant nephrosclerosis, MSK = medullary sponge kidney, CIN = chronic interstitial nephropathy

Pat. no.	RAT[a] no.	Sex	Age (y)	Diagnosis	Regular dialysis (mo.)	Kidney function[b] after RAT	Clinical neuropathy[c] before RAT	Comments
7	8	♂	41	PCK	3	70	x	
13	38	♂	29	GN	3	70–80	xxx	
35	3	♂	27	GN	21	55	—	
47	1	♀	28	GN	9	80	xxx	
87	42	♂	33	GN	2	70	xx	
89	52	♀	17	PCK	6	54–0	—	Acute rejection, uremia after 1 week
90	46	♂	43	PN	11	80	xx(x)	
91	37	♀	51	PN	6	50	x(x)	
97	47	♀	25	PN	3	60–0	x	Chronic rejection, died after 7 months
98	44	♀	34	GN	3	40	—	
100	48	♂	22	GN	2	90	—	
102	39	♀	26	MNS	5	30–0	xx(x)	Chronic rejection, died after 3 months
103	40	♀	21	PN	1	80	—	
106	43	♂	44	PN	1	100	xx	
109	51	♀	42	PN	0	40	x( )	Perf. gastric ulcer, died after 1 month
112	49	♀	23	MSK	2	110	(−)	
113	50	♂	22	Alport syn.	1	90	(−)	
117	41	♀	30	GN	0	50	(−)	
118	53	♀	37	CIN	9	<10–0	x	Oliguria, azotemia not eliminated, died
120	45	♀	22	PN	1	70	(−)	
123	2	♀	44	PN	0	65	—	
26	6	♂	25	PN	1	90	xx(x)	
7	56	♀	29	GN	0	85	—	
	57	♂	28	PCK	0	70–35	xx	Reversible hyperglycemia 2 months after RAT
31	4	♂	33	PN	3	65	x(x)	
132	5	♂	35	PN	2	55	xx	
134	7	♂	45	MNS	8	80	xx(x)	Acute hepatitis, died after 2 months

[a] 1–8 = Rigshospitalet, Copenhagen, 37–57 = Municipal Hospital, Århus.
[b] Creatinine clearance (ml/min) of the kidney transplant.
[c] — = absent, x = mild, xx = moderate, xxx = severe.

a kidney transplant from monozygotic twin donors. In the other 25 patients close relatives (parents in 17, siblings in 8) volunteered as donors. The observation period after transplantation covered 318 patient months (mean 12). Patient 118 was excluded from the follow-up study as the creatinine clearance (C_{cr}) of the graft never exceeded 10 ml/min. She died after 3 months. In all other patients the graft was immediately well functioning with elimination of azotemia within days, as judged from the serum concentrations of creatinine and urea. However patient 89 developed fulminant acute rejection episode with anuria. The graft, showing multiple severe haemorrhagic necroses, was removed and regular haemodialysis resumed. Another two patients (nos. 97 and 102) became primarily anazotemic, but the graft function gradually deteriorated after 3 and 1 months due to chronic rejection. They died after 7 and 3 months. Two patients (nos. 109 and 134) died after 1 and 2 months from extrarenal complications, both with well functioning grafts. Thus, the long-term course after transplantation could be followed in 21 patients (78%). C_{cr} of the graft was > 70 ml/min in 13 patients, 50–70 ml/min in 6, and 35–50 ml/min in 2. All patients, except the two with monozygotic twin donors, were given azathioprine and prednisone as permanent immunosuppressive treatment, and almost all were fully resocialized.

Neurological examination. The neurological course prior to transplantation in seven of the patients has been described in a previous paper (9). Another seven patients were followed during terminal renal failure for 2–4 months, while 13 were examined only once shortly before transplantation. The neurological examination also comprised a determination of the VPT on the pulp of the big toe (PH) and on the medial malleolus (MM). The method has been described in a previous paper (8). A complete muscular status was performed in selected

Table II. *Symptoms and signs of peripheral neuropathy before and after renal transplantation*

Pat. no.	Obs. time (mo.)	Symptoms[a] Pre	Post	Deep reflexes[b] Biceps	Triceps	Patellar	Ankle	Paresis-atrophies Arms	Legs	VPT[c] PH	MM	Hypesthesia/-algesia[a] Arms	Legs
13	31	xxx		−/+	−/+	−/+	−/−	xxx/x	xxx/x	50–50/50–50	49–50/37–30	x/−	xxx/x
47	29	xxx		−/+	−/+	−/+	−/−	xxx/x	xxx/x	50–50/50–50	50–50/20–20	xx/−	xxx/x
87	29		−			−/+	−/+	x/−	x/−	36–32/10– 6	27 25/10– 9		
90	28	xx	−	−/+	−/	−/+	−/+		(x)xx/−	45–31/28–31	15–23/20–20		x/−
91	6		−		−/+	(−)/+	−/+	(x)/−	x/−	20–19/13–11	17–19/20–19		
102	3†	xx	(x)			−/−	−/−	xx/x	xxx/xxx	1 19/18–17	14–15/16–11		(x)xx/x
106	14		−			−/+	−/+		x/−	38–44/27–20	38–26/19–18	x/−	x/−
109	2†					(−)/+	(−)/+	(x)/−	(x)/−	25–26/18–14	24–21/16–14		
126	20	xx	−			−/+	−/+		(x)xx/−	50–48/10–11	23–21/12–11		xx/−
130	26	xx	−			(−)/+	−/+			38–42/ 8– 9	21–32/11 12		
131	8								(x)x/−	20–20/11– 9	18–17/ 9– 9		
132	21		−			−/+	−/+		(x)xx/−	30–21/ 9–11	16–13/13–13	x/−	x/−
134	1†	xx	−				−/−	( )/−	xxx/(x)xx	40–42/28–40	21–23/20–24		

[a] − = absent, = mild, xx = moderate, xxx = severe.
[b] − = absent, + = present.
[c] Read values, 0–50 V, mean of three determinations, dx–sin/dx–sin.

patients, using 0–5 scale system (6). Nerve conduction studies before and after transplantation were made in 20 of the patients (10).

RESULTS

The material was divided into two groups according to the VPT on PH at the time of th transplantation. *Group I* (no or mild neuropathy) comprised 14 patients, ten females and four males, in whom VPT (PH) was normal. The average age was 28.1 years (range 17–44 years). *Group II* (moderate to severe neuropathy) comprised 13 patients, four females and nine males, in whom VPT (PH) was significantly elevated. The average age was 35.5 years (range 26–51). Regular dialysis treatment had been performed in 11 patients in each group and there was no difference in the duration of the treatment (mean 4 7 months, range 1–21).

Group I

Six of the 14 patients in this group had no symptoms or signs of clinical neuropathy before or after transplantation. Eight had mild neuropathy consisting of paresthesia in the toes in four absent patellar and ankle jerks in two, while another two patients had mild paresis of the dorsiflexion of the big toe, a reminiscence of a peroneal palsy which had recovered considerably during regular hemodialysis.

The observation period *after transplantation* ranged from 1 to 27 months (average 7.3). VPT remained normal throughout the observation period (Fig. 1). Paresthesia disappeared within 1 or weeks, and deep reflexes reappeared within 2 months. In one of the two patients with paresis, normal muscle power was regained within 3 months, the other (pat. 118) was not reexamined as uremia was not eliminated after transplantation.

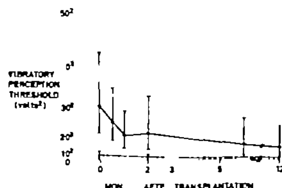


Fig. 1. VPT (PH) before and after renal transplantation. Upper curve = moderate to severe neuropathy 11 patients (two nos. 13 and 47 were excluded, as the vibration sense remained extinct throughout the observation period) Lower curv no or mild neuropathy 14 patients A erage values ±S D (data were transformed into log, V in order t obtain statistically normal distribution)





Fig 2. Patient 13. Severe atrophy and pareses of muscles in the hands and legs before renal transplantation. The strength of muscles in the hands was graded 0–2, in the forearm –4, and in the pper arm 4–5 In the legs, muscles distal to the knee were paralytic (grade 0), and in the thigh severely paretic (grade 1–4). (Compar pat. 47 Table III.) Three months after transplantation he was able to resume his ork. H could eat and drink unaided, use cutlery write, rise from chair climb stairs, and was able to alk, using arm crutches outdoors only

Group II (Table II)

Before transplantation 11 of the 13 patients in this group complained of paresthesia, dysesthesia, or motor restlessness. One or more of the deep reflexes examined were clearly weakened or absent in 12 patients. Muscular weakness and wasting in the legs were recorded in 12 patients, 7 of whom had also motor disturbances in the arms. Patients 13 and 47 had paralysis of muscles distal to the knee and several distal muscles in the arms were either paralytic or extremely paretic and atrophic. Proximal muscles were also clearly affected. Both patients were bedridden and unable to eat or drink unaided (Fig. 2). As stated above, all patients had elevated VPT on the PH, while the vibration sense was less severely impaired on the MM, the VPT PH/MM ratio ≥ 1 in all patients. Other sensory disturbances (hyp/anesthesia, -algesia) in the legs were present in 7 patients, 4 of whom had also sensory loss in the hands.

After transplantation Three of the 13 patients died 1–3 months after transplantation. All showed definite evidence of improvement, but the remission of the neuropathy was complete in only one. The other ten patients were followed for 6–31 months (average 21.2). The remission of the neuropathy had a very uniform course and led to complete recovery in eight of the ten patients.

Symptoms disappeared within the first days or weeks, but some patients described a new dysesthetic sensation in the legs and feet, which could persist for 1–2 years. This was characterized by a discomforting sensation of coldness or an

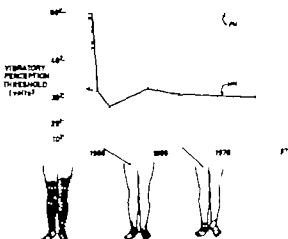


Fig 3. Patient 13, male 29 years old. Remission of sensory disturbances after renal transplantation (RAT). The curves show the VPT (PH, MM). The rapid return of normal cutaneous sensibility within the first months is illustrated below the graph. ☐ = hypesthesia, ☐ = anesthesia.

Table III. *Muscular strength in patient 47 (♀ 28 y) before and after renal transplantation (Jan. 30)*

0 = no contraction, 1 = trace, 2 = activ movement, gravity eliminated, 3 = active movement against gravity 4 = active movement against resistance, 5 = normal power (6)

Nerve	Muscle	Jan. 27 (d)	(s)	Apr 23 (d)	(s)	June 7 (d)	( )	Sept. 19 (d)	(s)	Dec. 17 (d)	(s)
Musculocutaneous	Biceps brachii	5	5	5	5	5	5	5	5	5	5
Radial	Triceps brachii	5	5	5	5	5	5	5	5	5	5
	Extensor digit.	5−	5	5	5	5	5	5	5	5	5
	Ext. poll. brev	5	5	5	5	5	5	5	5	5	5
	Ext. poll. longus	5	5	5	5	5	5	5	5	5	5
Median	Flexor digit. sublimes	3	3	5	5	5	5	5	5	5	5
	Flexor digit. profund.	2	3	5	5	5	5	5	5	5	5
	Abd. poll. brev	3	0	4	1	5	1	5	1	5	1
	Opponens poll.	2	0	2	0	3	0	5	1	5	1
Ulnar	Flexor carpi ulnaris		3			5	5				
	Abd. dig. V	1	0	0	0+	0	0	0	0	0	0
	Add. poll.	0	0	0+	0+	3−	0	5	0	5	0
Sciatic	Gluteus max.	2	2−	3	3	4	4	5	5	5	5
	Gluteus med. + min.	2	2+	3	3	4−	4	5−	5	5	5
	Biceps femoris	1	1	3	3	4	4	5	5	5	5
Femoral	Iliopsoas	4	3	5	5	5	5				
	Quadriceps	3	2	5−	5	5	5	5	5	5	5
Obturator	Adductores	2−	2	3−	3	4	4	5	5	5	5
Posterior tibial	Triceps surae	0	0	1	1	2−	2−	3−	3−	3	3
	Tibial post.	0	0	0	0	0	0	0	0	0	1
	Flexor digit.	0	0	0	0	0	0	0	0	0+	0
Common peroneal	Tibial ant.	0	0	0	0	0	0	0	0	0	0
	Ext. dig. brev	0	0	0	0	0	0	0	0	0	0
	Ext. hallucis	0	0	0	0	0	0			0	0

unpleasant *hyperesthesia* and *hyperalgesia* in previously hyp- or anesthetic areas. Thus, walking on uneven ground, sudden blows, or touching rugged or granular surfaces could be extremely painful.

The earliest objective evidence of remission was a significant decrease in the VPT within the first weeks (Fig. 1). After one month the average VPT (PH) was reduced to 46% of the pretransplantation value ($p < 0.001$) and during the first year a further reduction to 22% was recorded. In patients 13 and 47 the vibration sense on the big toe remained extinct for $2\,^1/_2$ years, while a considerable decrease was recorded on the MM within 2–3 months (Fig. 3). The decrease in VPT was accompanied by disappearance of other sensory disturbances. In three patients, in whom the proximal border of the hypesthetic area reached the knee or mid-thigh, normal cutaneous sensibility rapidly returned in a distalward direction during the first month after transplantation (Fig 3). Patients 13 and 47 never regained normal sensibility in their feet.

The reappearance of deep reflexes was more retarded. The biceps, triceps and patellar reflexes usually returned after 3–6 months, while the ankle jerks could be absent for up to two years after transplantation. In patients 13 and 47 they had not returned after $2\,^1/_2$ years. Six months after transplantation, muscular impairment was demonstrable in only three patients, who had severe muscular weakness and wasting prior to transplantation. Table III shows the pattern of recovery in patient 47. Most muscles of the arms and proximal muscles of the legs recovered rapidly and became clinically normal within the first year. Single paralytic muscles recovered partly while the muscular function never returned in most distal muscles in the legs and in single muscles in the hands during $2\,^1/_2$ years of observation. Note the disperse and asymmetrical distribution of paralytic muscles in the hands. However despite the incomplete recovery of the muscular function patients 13 and 47 became fully resocialized in their previous occupation as lawy[illegible] and as a housewife.

One patient showed a transient relapse or exacerbation of neuropathy about two months after a successful transplantation.

Patient 130

A 28-year-old man with polycystic kidneys. Two grandparents had diabetes mellitus and a brother died 20 years old of juvenile diabetes. Renal failure was detected 6 years before renal allotransplantation (RAT), but he did well until 5 months before RAT. Serum glucose concentrations one week before RAT were 850–1 020–840 mg/l, and ophthalmoscopy showed no microaneurysms.

Renal transplantation (May 15, 1968) was immediately successful. C_c of the graft was 75–100 ml/min, and the serum creatinine concentration was normal from the second postoperative day. Five weeks later a rejection episode resulted in reduction of the clearance to 30–35 ml/min and a rise in serum creatinine concentration to 25–30 mg/l. This level was maintained for the following two years. At the same time hyperglucosemia and glucosuria were detected. Insulin was administered from June 22 to Oct. 28, when he had a hypoglucemic attack. Since then the fasting serum glucose level has remained normal and the urine has been free of sugar.

Neurological examination. During the last three months of terminal uremia he complained of paresthesia in the toes and fingers and of pains in the calves, but he had no difficulty in walking. A clinical neurological examination five days before RAT is summarized in Table II. The sensory nerve conduction in the median nerve and the motor conduction velocity in the median and the common peroneal nerves were reduced. Electrophysiological signs of a neurogenic atrophy were present in the dig. brevis muscle. Three weeks after RAT motor action in arm and leg was unchanged, while the sensory nerve conduction in the median nerve had improved significantly. VPT had become reduced but a moderate weakness of the dorsiflexion of the toes was now present. Six weeks after RAT, i.e. at the time of the rejection episode, he developed severe peroneal palsy in the right leg with sensory loss in the foot. He had to wear brackets for three months, but after two months he gradually recovered. The gait was normal six months after RAT and after 10 months clinical signs had disappeared completely and VPT values had become normal. Sensory and motor nerve conduction velocities were now within normal range. This status was unchanged at the last examination 26 months after RAT.

DISCUSSION

A successful renal transplantation is a unique situation in the study of a metabolic neuropathy. The access to a kidney transplant from a living donor enabled timing of the neurological examination in close connection with the transplantation. The reestablishment of a normal kidney function resulted in rapid elimination of potentially neurotoxic agent(s), the obvious prerequisite for the remission of the neuropathy. Due to the uniform nephrological course after transplantation with only one primary failure of the graft, the remission of neuropathy could be precisely time-related to the date of the renal transplantation which was of particular value in the statistical analysis of numerical variables (e.g. VPT).

The present study has shown that the major part of the recovery from clinical neuropathy after a successful transplantation took place during the first 1–3 months. As previously demonstrated (9) the VPT was a most useful indicator of the clinical course. Within the first weeks VPT was significantly reduced preceding the remission of other sensory-motor functions. The remission was two-phasic with an early rapid phase and a late slow phase. Complete recovery was observed in all but four patients, two of whom died with evidence of *partial* remission. After 2 1/2 years another two patients presented permanent sequelae, comprising paralysis of distal muscles in the legs and of isolated muscles in the hands, absent ankle jerks, extinct vibration sense on the big toe, and hypesthesia of the feet.

This material exhibited the characteristic distribution of clinical neuropathy with a predominance of males in the upper part of the age range (11). The frequency (63 %) was lower than expected in terminal renal failure (5, 8, 11). This may be due to the fact that the donor problem was usually solved in advance, i.e. it was possible to select the optimal time for the transplantation before severe uremic complications became manifest. In addition, in three patients a considerable clinical improvement of neuropathy had been observed during long term dialysis treatment. The degree of affection covered the whole spectrum from normality to extremely severe polyneuropathy. It deserves attention, however, that most patients with no or mild neuropathy had slowed sensory and motor nerve conduction velocity, some even pronounced (10).

Irrespective of the presence of clinical neuropathy all patients experienced a sense of unprecedented well-being immediately after transplantation (1). In fact, many of them only then realized the degree of physical and mental incapacity during terminal uremia. The two-phasic course of recovery, as described by Dobbelstein et al. (3) was clearly demonstrable in patients with moderate to severe neuropathy. In the early

phase the rate of regression was indeed faster than anticipated. This was illustrated by the instantaneous and marked fall in VPT within the first weeks (see also Fig. 3 in a previous paper (9)). The clinical remission occurred in the opposite order to the development of neuropathy i.e. arms before legs, proximal before distal. A dissociation was suggested between the remission of sensory and motor signs. Within a few months sensory symptoms and signs either disappeared completely or became definitively demarcated, i.e. showing no further tendency to regress during the following two years. The remission of motor disturbances, on the other hand, initially showed a rapid functional improvement, which in less paretic muscles led to full restitution, but in more severely affected muscles was succeeded by a more protracted course of continued improvement, probably as the result of reinnervation. However muscles which were still paralytic after about 6 months apparently never recovered. In the upper extremity the final sequelae showed a peculiar random and asymmetrical pattern (Table III).

Transient or permanent relapses of neuropathy after renal transplantation have also been reported by others (1 3). They may be due to a severe and protracted reduction of the kidney function (1), a relapse of uremia, or to an increased susceptibility to pressure insults (especially the peroneal and ulnar nerves), which is probably common to most of these patients. I am familiar with at least two examples of the latter type. They both showed evidence of neuropathy prior to transplantation, both recovered, but slowly Close observation and prophylactic measures are clearly indicated in, e.g. prolonged bed rest. Neither of the two possibilities was suspected in the present case (pat. 130) The rejection episode resulted in a deterioration of the kidney function, but the C_{Cr} of the graft never passed below 30–35 ml/min, and this level was compatible with full clinical remission of neuropathy later in the course. The relapse was probably correlated with the diabetic reaction induced during the intense immunosuppressive treatment in this hereditarily afflicted patient. Immunosuppressive treatment per se does not seem to have any adverse neurological effects.

With due reservation for the limited number of patients in the present investigation, the following conclusions were suggested as to the *prognosis* of uremic neuropathy after a successful renal transplantation.

1) The prognosis is good if symptoms and signs are mild and moderate and also favorable in more severe cases, provided that a certain function is demonstrable before transplantation. 2) The prognosis seems dubious when sensory and motor functions are lost before transplantation, but definitely bad only when functional restoration has not been detectable during the first 6 months. This, however only applies to single neurological functions, not to the total clinical picture.

ACKNOWLEDGEMENTS

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

IX. *Recovery after Renal Transplantation Electrophysiological Aspects (Sensory and Motor Nerve Conduction)*

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Abstract. Sensory and motor nerve conduction parameters have been studied before and after successful renal transplantation in 22 patients, 13 of whom had moderate to severe clinical neuropathy The observation period ranged from 5 weeks to 31 months. The earliest post operative examination was made after 6.2±0.8 weeks, and 10 patients were studied for more than two years. Before transplantation general slowing of the nerve conduction was present. After transplantation the conduction velocity increased in all patients. The remission was two-phasic with an early rapid and late slow phase. The recovery was more rapid and complete in distal than in proximal segments, where the conduction velocity was still significantly slowed after two years. Sensory action potentials (median nerve) became resynchronized and the amplitude was normal after 6 (wrist) and 12 months (elbow). Signs of reinnervation of distal muscles were observed after one month The amplitude of muscle action potentials increased and in most patients the electromyogram at maximal effort became normal, though in severely affected muscles the recovery was incomplete or absent. The uniform degree of impairment and the pattern of recovery in distal and proximal nerve segments appeared incompatible with an ascending segmental demyelination and axonal degeneration as the only pathophysiological factor of uremic neuropathy

In most uremic patients the remission of clinical neuropathy is completed within 1–6 months after a successful renal transplantation (20). The peripheral nerve conduction also improves, although more slowly and less completely Chaumont et al. (7) and Funck-Brentano et al. (13) showed that the increase in the motor conduction velocity might continue for 1–2 years, and the distal motor latency apparently decreased in parallel Their data did not cover the initial phase after transplantation and comprised only 10 patients Dobbelstein et al. (9) drew attention to an early rapid and a late slow phase in the remission of clinical neuropathy and a similar pattern was seen in the recovery of the motor nerve conduction. This was not apparent from the study by Bolton et al. (4).

No detailed information is available as to 1) the early changes in the nerve conduction parameters after transplantation, 2) the relative improvement in sensory and motor nerves or in distal and proximal nerve segments, or 3) the changes in sensory nerve action potentials. It was the purpose of the present report to study these aspects. Electrophysiological examinations were made shortly before, and repeated as early as possible after transplantation. Further reexaminations were made for up to 2 $/_2$ years in a sufficient number of patients to permit a statistical analysis of the early and late course of recovery and to outline permanent sequelae. From these data, pathophysiological aspects of the remission will be discussed.

MATERIAL AND METHODS

This study comprises 22 patients, 8 females and 14 males, 21–44 years of age (average 32.1). Before transplantation 9 patients had no or mild clinical neuropathy (i.e. symptoms and/or single clinical sign), while 13 had moderate to severe clinical neuropathy In previous paper (20) the clinical course after transplantation was described for 20 of the 22 patients.

Seventeen patients were examined shortly before transplantation Reexamination after transplantation was not possible in two, due to sudden death and irreversible failure of the graft Four patients were reexamined once, 5 weeks to 11 months after transplantation, while 11 were

Table I. *Distal motor latency (msec) and motor and sensory nerve conduction velocity (m/sec) before and after renal transplantation (RAT). Mean ± S.E. (S.D.) (no. of pats.)*

Segment	Normal	Pre RAT	Post RAT 6 weeks	Post RAT 6 months	Post RAT 1 year	Post RAT 2 years
Distal motor latency						
Wrist abd. poll. brev	3.2 ± 0.09 (0.5) (34)	3.7 ± 0.17 (0.7) (17)	3.3 ± 0.19 (0.8) (16)	3.3 ± 0.21 (0.7) (11) –	3.4 ± 0.11 (0.4) (13)	3.4 ± 0.16 (0.4) (8)
Ankle ext. dig. brev	4.1 ± 0.08 (0.5) (42)	6.0 ± 0.32 (1.2) (14)	5.4 ± 0.29 (1.1) (15)	4.6 ± 0.34 (1.0) (9)	4.9 ± 0.36 (1.2) (11)	5.0 ± 0.25 (0.7) (8)
Motor conduction velocity						
Elbow wrist	62.8 ± 0.70 (4.2) (34)	45.4 ± 2.38 (9.8) (17)	50.2 ± 2.32 (9.3) (16)*	53.3 ± 2.68 (8.9) (11)	54.9 ± 2.37 (8.6) (13)	54.0 ± 1.58 (4.5) (8)
Cap. fibulae ankle	50.0 ± 0.54 (3.5) (42)	33.8 ± 2.20 (8.2) (14)	36.8 ± 2.20 (8.5) (15)	40.7 ± 2.29 (6.9) (9)	41.7 ± 2.48 (8.2) (11)	57.2 ± 2.06 (5.8) (8)
Sensory conduction velocity						
Digit I wrist	54.2 ± 0.64 (3.9) (34)	41.4 ± 1.18 (4.9) (17)	45.7 ± 0.89 (3.6) (16)	51.2 ± 1.84 (6.1) (11)	51.2 ± 1.00 (3.6) (13)	51.4 ± 1.08 (3.1) (8)
Wrist elbow	66.4 ± 0.77 (4.5) (34)	48.7 ± 1.84 (7.6) (17)	54.4 ± 1.93 (7.7) (16)	59.4 ± 2.22 (7.4) (11)	60.8 ± 1.70 (6.1) (13)	60.5 ± 0.80 (2.3) (8)

Significance of difference from the normal mean value – $p > 0.10$, $p < 0.05$, $p < 0.01$ $p < 0.001$

reexamined twice or more during 8–31 months of observation. Five patients were only examined after the transplantation. Three of them were followed from 4 weeks to 14 months, while two were followed from 1 to 2 years.

A total of 73 electrophysiological examinations were made. These were divided into five groups: (a) 17 examinations 12.7 ± 3 days (mean ± S.E.) *before* transplantation, (b) 17 examinations 6.2 ± 0.8 weeks (range 3–12) *after transplantation*, which was the earliest possible moment after the operation, (c) 11 examinations after 6.6 ± 0.5 months; (d) 13 examinations after 12.4 ± 0.4 months; (e) 10 examinations after 26.1 ± 1.1 months. The latter group only comprised the most affected patients. In addition, five patients were examined once each inbetween these periods.

The examination comprised. 1) *The sensory conduction velocity* in the median nerve, stimulating digit 1 and recording at the wrist (distal segment) and at the elbow (proximal segment). 2) The *distal latency* in the median *and common peroneal nerves, stimulating* at the wrist and at the ankle and recording from the abd. poll. brevis and the ext. dig. brevis muscles, respectively. 3) The *motor conduction velocity* in the median and common peroneal nerves between elbow and wrist, and capitulum fibulae and ankle, respectively. 4) The shape and peak-to-peak amplitude of the *evoked sensory nerve action potentials* at wrist and elbow and of *evoked action potentials* in the abd. poll. brevis and the ext. dig. brevis muscles. 5) The amplitude and the shape of the *electromyographic (EMG) pattern at maximal voluntary contraction* of the abd. poll. brevis and the ext. dig. brevis muscles.

Sensory fibers in digit 1 were stimulated through ring electrodes, otherwise needle electrodes were used throughout for stimulation and recording. Further details about the procedure have been reported previously (5, 17).

The extremities were heated prior to and during the examination and *temperatures* were recorded proximally and distally near the nerve with a thermoneedle. Temperatures averaged 35.2 ± 0.2°C (median nerve) and 33.5 ± 0.?°C (common peroneal nerve). Variations from one series of examination to another are shown in Table II.

Normal values were obtained from 34 normal persons, 20–50 years of age (median nerve (21)) and 42 persons, 20–60 years of age (common peroneal nerve). Age and sex variations were neglected as each patient served as his own control.

The *statistical analysis* in paired observations has been described elsewhere (19).

RESULTS

Sensory and Motor Nerve Conduction (Tables I and II)

Before transplantation

In one of the 17 patients the nerve conduction had become normal during 21 months of regular hemodialysis (19). Another two patients had conduction velocities within the normal range in one or more of the four nerve segments examined.

Median nerve. The average distal motor latency was increased, 3.7 ± 0.17 msec ($p < 0.05$), but the

Table II. *Average difference in distal motor latency (msec), conduction distance (mm), motor and sensory nerve conduction velocity (m/sec), and mean temperature near the nerve (°C) after renal transplantation. Average difference* $\bar{X}_D \pm S.E.$ *(no of pats.)*

	Interval			
	Pre RAT–6 weeks	6 weeks–6 months	6 months–1 year	1 year 2 years
Distal motor latency				
Wrist abd. poll. brev	−0.35±0.15 (13)	−0.07 (10)⁻	0.04 (7)⁻	−0.03 (7)⁻
(conduction distance)	0 5 (13)⁻	3.5 (10)⁻	3.7 (7)⁻	4.3 (7)⁻
Ankle ext. dig brev	−0 40±0.28 (11)⁻	−0.46±0.24 (9)	0 22 (5)⁻	−0 34±0.28 (6)
(conduction distance)	9 0 ±3.3 (11)*	−0.25 (9)⁻	−1.6 (5)⁻	−1 4 (6)⁻
Motor conduction velocity				
Elbow – wrist	4 0 ±1 14 (13)	4 8 ±1.22 (10)*	2.43+0.72 (7)	3 86±1.21 (7)
Cap. fibulae – ankle	3.45±1 16 (11)	6.11±1.38 (9)**	1.6 ±1.55 (5)⁻	1.67±0 80 (6)ᶠ
Sensory conduction velocity				
Digit I – wrist	3.92±0.90 (13)	4.7 ±0.91 (10)	2.14±1.28 (7)⁻	1.86±1.58 (7)⁻
Wrist elbow	5.23±0.82 (13)	4.7 ±1.14 (10)	2.14+1.08 (7)ᶠ	2.71±1 87 (7)⁻
Mean temperature				
Median nerve	0.58±0.33 (13)⁻	0 20±0 22 (10)⁻	0 33+0.27 (7)⁻	0 43±0.24 (7)⁻
Common peroneal nerve	−0.38±0.36 (11)⁻	0.8 ±0.27 (9)	−1.58±0.46 (5)	1.2 +0 56 (6)⁻

Significance of difference from zero $p > 0.10$, ( ) $0\,10 > p > 0\,05$, $p < 0\,05$ $p < 0\,01$, $p < 0\,001$

values exceeded the upper normal limit in only 4 of the 17 patients, whereas the motor conduc tion velocity (elbow–wrist) was significantly re duced in 14, average 72% of the normal ($p < 0.001$). The average sensory conduction velocity in distal and proximal segments was reduced to 76 and 73% of the normal, corresponding to a reduction of 13 and 18 m/sec, respectively ($p < 0.001$).

Common peroneal nerve The ext. dig. brevis muscle was inexcitable in three patients. Of the remaining 14 patients the distal motor latency was significantly increased in 11 a erage 6.0± 0.32 msec ($p < 0.001$). The motor conduction velocity was reduced to 68% of the normal, aver aging 33.8±2.2 m/sec ($p < 0.001$).

After renal transplantation

The average distal motor latency became normal within 6 weeks and 6 months after transplanta tion in the median and common peroneal nerve, respectively There was a significant improvement of the conduction velocity in the proximal motor nerve segments too but the average values had not returned to normal after on or two years. (In the common peroneal nerve the decrease after the first year (Table I) was only pparent, as the last examination comprised the most affected patients only) As to the sensory conduction in the median nerve, the average conduction velocity became normal after six months in the *distal* seg ment, while the conduction velocity in the *prox*

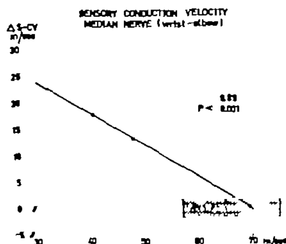


Fig 1 Maximal increase in nerve conduction, ΔS-CV (m sec), one year after renal transplantation related to the pretransplantation conduction velocity in 13 patients. T patients were excluded as the last examination was performed 5 and 10 weeks after transplantation. □– 95 of interindividual variation (horizontal xis) and 95% of intraindividual variation (vertical axis) in normal persons.

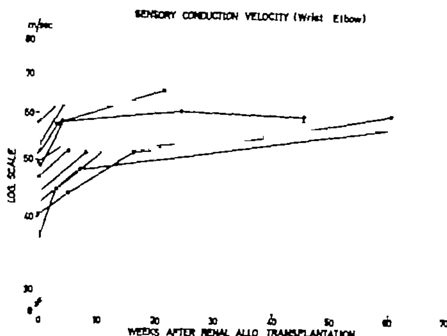


Fig. 2. The two-phasic course of recovery of sensory nerve conduction *after renal transplantation.* Individual curves in ten patients in whom the first examination was performed on average 4.6 weeks after transplantation. Five patients were excluded from the graph, because they were examined only once after transplantation (four patients), or because the first postoperative examination was not performed until 12 weeks after transplantation (one patient).

imal segment was still significantly reduced at the 1% level after one and two years (Table I).

During the first year the greatest improvement was seen in the patients most *severely affected* prior to transplantation (Fig. 1) but it is noteworthy that three of four patients with conduc velocities within or slightly below the normal showed increases exceeding the expected raindividual variation.

The average *individual differences* within each of the four intervals appear from Table II. When comparing distal motor latencies, differences in the conduction distance had to be considered. The decrease of the latency from the wrist to the abd. poll. brevis muscle could not be accounted for by changes in the conduction distance. However the fact that the decrease in the latency from the ankle to the ext. dig. brevis muscle observed during the first 6 weeks was comparatively small and insignificant was at least partly explained by the significantly longer conduction distance at the second examination. After 6 months there was no systematic change in the distal motor latencies.

The average increase of the sensory and motor conduction velocities in the median nerve could not be attributed to temperature variations, but in the common peroneal nerve the increase and decrease in the mean temperature during the second and third observation period may have influenced the changes in the motor conduction velocities, which are thus probably over- and underestimated, respectively. The motor conduction *velocities showed a continuous increase* throughout the observation period. The same tendency was seen in sensory segments of the median nerve, although the average increase during the last two observation periods was not significantly different from zero.

The course of recovery as a function of time consisted of two separate phases (Fig. 2). The actual rate of the *initial rapid phase* could not be defined due to the fact that the individual patient was only examined once during the first postoperative weeks. The *second slow phase* was only clearly present in patients with moderate to severe slowing of the nerve conduction prior to transplantation. The slope was almost parallel in individual patients and the increase continued until the conduction velocity reached normal levels.

The Sensory Nerve Action Potential (Table III)

The amplitude of the sensory action potentials was significantly reduced *before* transplantation ($p < 0.001$). At the elbow 11 patients had amplitudes below the lower normal limit, while this occurred in only 5 patients in potentials recorded at the wrist. The amplitude became normal after

Table III. *The amplitude (log μV) of sensory nerve action potentials, mean ± S.E. (S.D.), recorded at the wrist and the elbow following stimulation of digit I. Normal persons and uremic patients before and after renal allotransplantation (RAT)*

			After RAT			
	Normals ($n=60$)	Before RAT ($n=16$)	6 weeks ($n=15$)	6 months ($n=11$)	1 year ($n=12$)	2 years ($n=8$)
Wrist	1.48±0.03 (0.21)	1.11±0.06 (0.25) $p<0.001$	1.17±0.08 (0.29) $p<0.001$	1.39±0.06 (0.20) $p>0.10$	1.48±0.05 (0.18) $p>0.10$	1.47±0.10 (0.29) $p>0.10$
Elbow	1.03±0.02 (0.19)	0.46±0.07 (0.28) $p<0.001$	0.60±0.09 (0.32) $p<0.001$	0.83±0.05 (0.18) $p<0.005$	0.92±0.06 (0.21) $0.10>p>0.05$	1.02±0.05 (0.15) $p>0.10$

6 months at the wrist and after 1 year at the elbow. The longitudinal course in patient 47 (Fig. 3) illustrates the difference between the two recording sites in the degree of affection and the rate of recovery. This could not be attributed to differences in the electrode position relative to the nerve or in the stimulus strength, both of which may influence the amplitude. On the other hand, the increase in amplitude coincided with a decrease in the temporal dispersion and a resynchronization of the action potentials, as illustrated in Fig. 4.

The Evoked Muscle Action Potential and the EMG Contraction Pattern at Maximal Voluntary Effort (Table IV)

Abd. poll. brevis muscle. With two exceptions (pats. 13 and 47) the evoked muscle action potentials had a normal shape before transplantation. Within the first 6 months after transplantation the mean amplitude increased from 16.8 mV (10–27 mV) to 22.6 mV (13–37 mV), the increase averaging 6.2±2.3 mV, $p<0.05$. The EMG at maximal effort showed an interference pattern. After transplantation the average amplitude increased from 2.7 mV (1.5–5 mV) to 3.9 mV (1.5–10 mV).

In patients 13 and 47 the evoked muscle action potential before transplantation was highly polyphasic with an amplitude of 0.5 and 0.1 mV. The EMG at maximal effort showed discrete activity with reduced amplitude (0.4 mV). After transplantation the muscle action potential amplitude increased considerably within the first six months, but remained about 6 and 3 mV for the following two years. As shown in Fig. 5 the evoked response in the abd. poll. brevis muscle was at first severely polyphasic with a duration of about 20 msec, but with a normal latency (4 msec). The potential later became less irregular and of shorter duration following stimulation at the wrist, while the duration was considerably increased and the potential very irregular following stimulation at the elbow after 1–2 years. The EMG pattern at maximal effort returned to normal after $^1/_2$–1 year.

Ext. dig. brevis muscle. Before transplantation, the amplitude of the evoked muscle action potential was normal in seven patients (4–11 mV) and slightly reduced in three (2.8–3.0 mV). In five of these 10 patients the evoked response was polyphasic, and in four the EMG at maximal effort showed discrete activity with a reduced amplitude (0.3–1 mV). After transplantation, electrophysiological signs of impaired muscular function disappeared in all 10 patients and the evoked potential amplitude increased significantly ($p<0.05$, Table IV).

The ext. dig. brevis muscle was completely denervated in three patients and in the remaining four the amplitude of the evoked potential was very low (0.1–0.4 mV). It was difficult to find an action potential and the EMG at maximal effort often showed silent areas or at most discrete activity of very low amplitude (0.1 mV). Two of the seven patients were not reexamined after transplantation, and in another two the muscle remained inexcitable for 2 $^1/_2$ years. In the last three patients, changes in the evoked potential closely resembled those illustrated in Fig. 5. The EMG at maximal effort became normal in amplitude, although in patient 132 there was no marked increase in the number of active

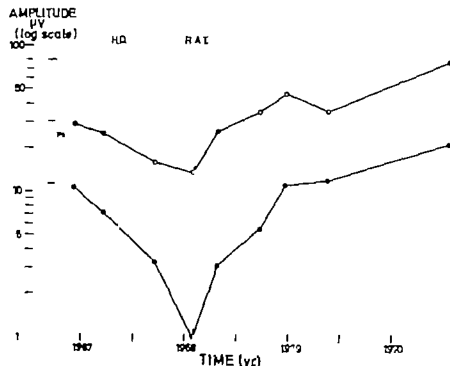


Fig. 3. The amplitude, μV (log scale) of sensory action potentials (median nerve) recorded t the wrist (o) and the elbow ( ) following stimulation of digit 1 in patient 47 female, 28 years old, during progressive renal failure and after enal transplantation. *H.D* = regular hemodialysis, *R.A T* = renal allotransplantation.

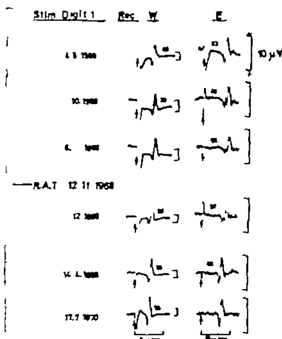


Fig. 4 Evoked median nerve potentials by stimulating digit 1 recorded from the wrist (*W*) and the elbow (*E*) during progressive chronic renal failure and after renal transplantation (*R.A T*) in patient 126, male, 25 years old. Arrow = stimulus artefact. Figure above each potential = the conduction velocity (m/sec) in the distal (digit 1–wrist) and proximal (wrist–elbow) segments of the median nerve. Note the desynchronization and resynchronization of the action potentials at the elbow

motor units as indicated by the pattern of discrete activity in the ext. dig. brevis muscle after 21 months (Fig. 6). Another characteristic finding was the great variability in the amplitude of the contraction pattern from one area to the other and that discrete activity was sometimes recorded side by side with areas showing an interference pattern. This description also applies to the anterior tibial muscle, which was examined in selected patients only

DISCUSSION

The impairment of the peripheral nerve conduction before transplantation was comparable with that recorded prior to regular hemodialysis (19). The two series differed in that most patients in the present series had been regularly dialyzed for some time. Only a few patients had low normal conduction velocities in one or more nerve segments, but the significant and lasting increase after transplantation showed that these values were pathological for the individual patients, reinforcing the statement that slowed nerve conduction is a general and generalized phenomenon in patients with terminal renal failure (18).

The distal motor latency rarely exceeded the normal range of variation in contrast to the con-

Table IV *Evoked muscle action potential (E–MAP) and EMG pattern at maximal voluntary contraction of the extensor digit brevis muscle before and after renal allotransplantation (RAT)*

		Before RAT				After RAT			
		E–MAP		EMG max. contract.		E MAP		EMG max contract.	
Pat. no.	Obs. time (mo.)	Amplitude[a] (mV)	Shape[b]	Pattern	Amplitude[d] (mV)	Amplitude[a] (mV)	Shape	Pattern	Amplitude[d] (mV)
7	2	2 8	P	II	1.5	9	N	II	2
13	31	0[e]	—	—	0	0[e]	—	—	0
35	3	11	N	I	2.5	18	N	I	4
47	29	0[e]	—	—	0	0[e]	—	—	0
87	29	4	P	III	1 0	6	N	I	2.5
90	28	0[e]	—	—	0	5	N	I	3
106	15	3	N	III	0 3	38	N	I	7
109	1	10	N	III	0 6	7	P	II	2
113	27	9	N	II	2.5	34	N	I	6
118	—[f]	0 2	?	III	0.1	—	—	—	—
123	11	3	N	I	2.0	10	N	I	2
126	20	0.1	P	III	0.1	2	P	I	1.5
127	11	11	P	I	2 0	16	N	I	3
130	26	11	P	II	4 0	10	N	I	4
131	8	4	P	III	0.9	10	N	I	
132	21	0 2	?	III	0.1	3 4	N	III	3
134	—[f]	0 4	P	III	0.1				

[a] Normal >3.5 mV
[b] N (normal) < 4 phases, P (polyphasic) >4 phases, ? = atypical, probably arising from one or few motor units.
[c] I = interference pattern, II = reduced interference pattern, III discrete activity —= no activity
[d] Normal >1.5 mV
[e] Inexcitable.
[f] Not reexamined.

duction velocity in proximal segments of motor nerves (17 19). This also appears from other studies (8, 13 15 23) The sensory conduction velocity in distal and proximal segments of the median nerve was affected to the same degree. After transplantation the distal motor and sensory nerve became normal within 6 months, whereas the average conduction velocity in prox imal segments of motor and sensory fibers was still significantly reduced after 2 years.

The recovery of the nerve conduction after a successful renal transplantation followed a two-phasic course (9). In mildly affected nerve segments the conduction velocity became normal within a few months, while more severely affected nerves improved considerably During the second, slower phase the changes in conduction elocity indicated a gradual decrease in the rate of improvement. The duration of the remission was mainly dependent on the severity of the preopera tive affection, and the curves in individual patients were surprisingly parallel, suggesting a common genesis of the restitution.

The remission of clinical neuropathy was also two-phase, but the course was considerably faster (20). Thirteen of the 22 patients had moderate to severe clinical neuropathy which disappeared completely within 1–6 months in all but three patients, i.e. long before the remission of electrophysiological findings. After one year clinical findings were only present in two patients, while e.g. the motor conduction velocity in the legs was considerably below normal in about half of the patients after 1–2 /2 years. Moreover in some of the patients there had been no sign of electrophysiological improvement during the last year of observation.

Before transplantation the desynchronization and the reduction in the amplitude of sensory action potentials were more pronounced at the elbow than at the wrist (17), and the return to normal was considerably slower at the elbow This

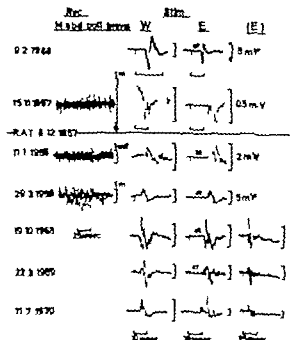


Fig. 5. Evoked muscle action potentials recorded from the abd. poll. brevis muscle after stimulation of the median nerve at wrist (*W*) and elbow (*E*) in patient 13 male, 79 years old. When not otherwise indicated, vertical brackets = amplitude = 5 mV. The potentials below to the right were recorded at 2.5 msec mm in order to show the decrease in potential duration. Figure above each potential = motor nerve conduction, elbow–wrist, m/sec. The extreme left column shows the EMG contraction pattern maximal voluntary effort. Note the rapid normalization pattern and amplitude within 5 weeks after the operation.

parallels the difference in the rate of recovery of the conduction velocity in the distal and proximal segments. The increase in amplitude accompanied a resynchronization and a decrease in the temporal dispersion of the action potential, indicating that the recovery also comprised more slowly conducting fibers. This picture was also seen in patients 13 and 47 with very severe polyneuropathy also in the median nerve area, probably the clearest indication of the reversible nature of impaired nerve conduction in uremic neuropathy.

The distal muscles in the upper and lower extremity appeared electrophysiologically normal prior to transplantation in most patients, i.e. also in patients with marked slowing of the motor conduction velocity (14). A general feature after transplantation, however, was an early increase in the amplitude of the evoked potential and of the EMG at maximal effort. This may reflect reactivation of previously blocked motor units, improved synchronization of the response, or an increase in the transmembrane potential difference after elimination of the uremic intoxication. The regeneration of severely affected muscles was absent in two patients and considerably more retarded and incomplete in others in keeping with the clinical picture (20). This suggests that a long-standing block of nervous impulses to the muscle may result in an atrophy which is not or only partly reversible.

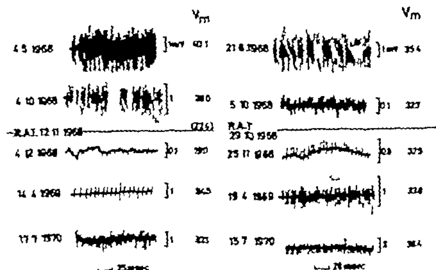


Fig. 6. Changes in the EMG contraction pattern at maximal voluntary effort (ext. dig. brevis muscle) during terminal renal failure and after renal transplantation (*R.A.T.*). V_m = motor conduction velocity (m/sec) in the common peroneal nerve (capitulum fibulae–ankle). In pat. 126 male, 25 years old (to the left), the figure within parentheses refers to the preoperative examination (6 11 1968), which unfortunately did not comprise the maximal contraction pattern. Note the pattern of decreased activity but with normal amplitude in pat. 132, male, 35 years (*to the right*), 21 months after transplantation.

Pathophysiological aspects

The restored renal function (increased glomerular filtration rate) not only results in the elimination of excess urea and creatinine within days, but probably also in the clearance of as yet undefined neurotoxic substance(s) of higher molecular weight (15). This may have two implications: 1) a normalization of the membrane function in nerve axons and muscle cells, and 2) a remyelination—maybe also regeneration—of damaged nerve axons, due to a restored Schwann cell function.

The rapid disappearance of sensory and motor symptoms and the marked fall in the VPT during the first 1–_ weeks are difficult to explain on a morphological basis (20). These findings may suggest a rapid improvement in the membrane function in nerve axons and muscle cells, irrespective of persistent structural changes. It is more difficult to assess whether a restored membrane function alone also accounts for the early changes in the nerve conduction parameters. The interval between the operation and the first electrophysiological examination in this study was probably long enough to make possible a reconstitution of the nodes of Ranvier of the myelin sheaths, which may also be responsible for the observed early electrophysiological findings. The late and protracted remission of the peripheral nerve function, on the other hand, probably only reflects regenerative changes in nerves and muscles, since it may be assumed that the membrane function has already been restored and remains normal provided that a normal graft function is preserved.

As previously discussed (17) ascending segmental demyelination and axonal degeneration ha e become widely accepted as the morphological basis of uremic neuropathy. Cavanagh and co-workers (2, 6) studied ultrastructural changes during experimental demyelination and remyelination. Allt (1), summarizing their findings, showed that the earliest stage in the regeneration was a reconstitution of the node of Ranvier demonstrable only 22 days after the induction of experimental demyelination. In slightly demyelinated segments the node was reconstituted by an extension of myelin lamellae from the original Schwann cell. In extensively demyelinated segments, new short intercalated myelin internodes were formed by new Schwann cells, resulting in more nodes of Ranvier per unit length of the axon than before demyelination. Coincidentally but more slowly apposition of new myelin lamellae took place by a process similar to that of primary myelinogenesis (16). In the latter the apposition of new myelin lamellae in the myelin sheath has been shown to increase as an exponential function of time (12).

This pattern of regeneration from partial demyelination appears compatible to observations in this study: the early increase in the conduction velocity after transplantation may reflect a reconstitution of the nodes of Ranvier increasing the current density at the node during depolarization. The secondary slow increase in conduction velocity in more severely affected nerves could be the result of a thickening of the internodal myelin sheath, increasing the ohmic resistance and hence the current density at the node (24). The permanent reduction of the conduction velocity in the most severely affected nerves would be the expected result of the formation of intercalated myelin internodes, which might delay the impulse propagation. A regeneration of the nerve axon should also be considered as a possible counterpart of the late increase in the conduction velocity.

However, in the literature detailed information about the pathoanatomical findings mainly reflects terminal renal failure *before* transplantation and almost exclusively concerns the sural nerve (10, 25). Thus, if the recovery of the conduction velocity in the median nerve is taken as evidence of regeneration, histopathological abnormalities in this nerve should be expected to a far greater extent than assumed from the few light-microscopic studies reported (3, 11). It is also conflicting that the recovery after transplantation was more protracted and less complete in proximal than in distal nerve segments. This might suggest that degeneration is also more pronounced in proximal than in distal segments, which evidently contradicts the concept of an ascending demyelination.

In conclusion this longitudinal study has shown that electrophysiological signs of peripheral nerve dysfunction in uremia disappear after a successful renal transplantation in the majority of patients in keeping with the remission of clinical neurological manifestations. The rapid increase in the nerve conduction velocity within weeks demonstrates the superiority of a successful renal trans-

plantation as compared to regular hemodialytic treatment in relieving uremic manifestations, as also emphasized by Bolton et al. (4). The two-phasic increase of the conduction velocity is probably the most interesting finding, although the pathophysiological significance is still obscure. The electrophysiological pattern of recovery may reflect morphological regeneration of the nerve fiber and its sheath, or a normalization of the axon membrane function, or both. However the uniform pattern of impairment and recovery in different nerves and segments of nerves seems incompatible with the general concept of pathoanatomical lesions in uremic nerves. For this reason an experimental study of changes of the nerve conduction during and after selective impairment of the axon membrane function may provide further information relevant to the subject (22).

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COMPARISON OF AMPICILLIN AND NALIDIXIC ACID IN THE TREATMENT OF URINARY TRACT INFECTIONS CAUSED BY E. COLI

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Abstract. A comparative study of ampicillin and nalidixic acid in short-term treatment of urinary tract infections caused by E. coli has been carried out in a series of female out-patients. Twenty-six patients were treated with ampicillin at a daily dosage of 2 g and 41 with nalidixic acid at a daily dosage of 4 g. Duration of treatment was 14 days in both groups. The frequency of primary resistance of E. coli to ampicillin was 13/67 (19%), whereas primary resistance to nalidixic acid was not found. Development of secondary resistance to ampicillin during treatment did not occur. Secondary resistance to nalidixic acid developed in only one of the 41 cases. In acute infections the cure rate was 14/15 (93%) in the ampicillin group and 18/19 (95%) in the nalidixic acid group. In patients with chronic infections the cure rates were 9/11 (82%) and 17/22 (77%), respectively. In contrast to previous reports, development of secondary resistance to nalidixic acid was not common. The rather favourable results obtained with nalidixic acid must be weighed against a relatively high frequency of side-effects in patients treated with this drug.

Both ampicillin and nalidixic acid have been used for about ten years in the treatment of urinary tract infections. An extensive literature exists concerning the use of these two drugs, but to our knowledge no formal comparative study of them has been reported. The literature conveys the impression that primary resistance of E. coli to ampicillin is more often encountered than primary resistance to nalidixic acid, although the frequencies of primary resistance to the two drugs vary widely. Nalidixic acid has been less favoured in use than ampicillin because in some studies a rapid development of secondary resistance to nalidixic acid during treatment has been reported (2, 3, 8, 10, 20, 23).

In the following we report observations concerning the occurrence of primary resistance to ampicillin and nalidixic acid in E. coli strains isolated from a series of female out-patients with urinary tract infections. Furthermore we report the results of a comparative study with ampicillin and nalidixic acid in the short-term treatment of urinary tract infections caused by E. coli.

PATIENTS AND METHODS

The series comprised 67 women, aged 16–77 years (mean 39), who had been referred by private practitioners to the Medical Out-patient Department of the University Central Hospital, Helsinki, because of urinary tract infection. The presence of E. coli infection was verified by a quantitative urine culture (10^5 colony count/ml) and by the finding of increased number of leukocytes in the urinary sediment. Twenty-six patients were treated with ampicillin at a dosage of 0.5 g four times a day and 41 received nalidixic acid at a dosage of 1.0 g four times a day. The duration of treatment was 14 days in both groups.

Patients were allocated randomly to the two treatment groups. However, patients who gave a history of penicillin or ampicillin allergy and therefore could not be treated with ampicillin, were treated with nalidixic acid. Similarly four patients in whom the referring physician already had found an ampicillin-resistant E. coli strain were treated with nalidixic acid. Thus 27 patients were treated with ampicillin and 43 with nalidixic acid. The treatment of one patient in the ampicillin group and of two patients in the nalidixic acid group had to be discontinued owing to side-effects. Hence 26 patients treated with ampicillin and 41 treated with nalidixic acid finally formed the comparison groups in the treatment trial.

While acknowledging the difficulties in dividing urinary tract infections into categories as regards their duration and pattern of recurrence, the infections in the present study were divided into "acute" and "chronic" infections on the basis of the following criteria. The infection was considered acute 1) if it was the first clinically manifest episode or 2) if a patient who gave a history of only one previous urinary tract infection the interval between the two episodes exceeded one year. All other infections were regarded as chronic. By

plantation as compared to regular hemodialytic treatment in relieving uremic manifestations, as also emphasized by Bolton et al. (4). The two-phasic increase of the conduction velocity is probably the most interesting finding, although the pathophysiological significance is still obscure. The electrophysiological pattern of recovery may reflect morphological regeneration of the nerve fiber and its sheath, or a normalization of the axon membrane function, or both. However the uniform pattern of impairment and recovery in different nerves and segments of nerves seems incompatible with the general concept of pathoanatomical lesions in uremic nerves. For this reason an experimental study of changes of the nerve conduction during and after selective impairment of the axon membrane function may provide further information relevant to the subject (22).

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As regards the occurrence of primary resistance of E. coli strains to nalidixic acid, it has been found to be rather low 3–16% (1 5 13 17 18, 19 24), and in some series has not occurred at all (20). In one series, however, primary resistance to nalidixic acid was reported in 47% of E. coli strains (7). Studies from several centres, in which the evolution of bac terial resistance patterns has been carefully monitored for several years, have not revealed any distinct change of the primary resistance of E. coli to nalidixic acid (4 9 12, 15).

In the present series the short-term results obtained in the treatment of acute urinary tract infections caused by E. coli were good both with ampicillin and nalidixic acid. In the series of patients with chronic E. coli infections the short-term results obtained with both drugs were also fairly good. Since patients, in whom the referring physician had already found primary resistance of E. coli to ampicillin, were not treated with this drug, the effectiveness of ampicillin apparently was overestimated to some extent in the present series. A factor which may have contributed to the favourable results obtained with both ampicillin and nalidixic acid is the relatively high dosage of both drugs employed in the present study

As regards the development of secondary resist ance to ampicillin and nalidixic acid during the 14-day treatment, the latter was not inferior to the former considering that secondary resistance to nalidixic acid occurred in only one of the 41 patients. This finding differs from the experience in other studies, in which development of secondary resist ance of E. coli t nalidixic acid has been reported to occur rapidly and rather frequently In some of the earlier studies in which secondary resistance was found to develop frequently the dosage of nalidixic acid was smaller than that used in the present study (10).

Regarding the occurrence of E. coli strains resistant to ampicillin and nalidixic acid in patients whose follow-up examination 2–10 months after the primary treatment revealed recurrent infections caused by E. coli, our experience is based on a few cases of such infections only However all those four patients primarily affected with an E. coli strain sensitive to ampicillin, and who later had a recur rence of infection, then carried an E. coli strain re sistant to ampicillin. On the other hand, one of the two patients primarily treated with nalidixic acid presenting a recurrence of infection at the follow-up examination also had an E. coli strain resistant to this drug.

Penicillin allergy is nowadays rather common its prevalence in the adult population of Finland has been estimated to be about 8 (E. Klemola, per sonal communication). It is worth noting that Sha piro et al. (25) have reported allergic skin manifestations to occur twice as often with ampicillin as with other penicillins. The absence of allergic reac tions in the ampicillin group in this study is ex plained by the exclusion of patients with a known history of penicillin allergy In two of the 43 patients treated with nalidixic acid an allergic reaction developed during the treatment.

One of the patients treated with ampicillin showed a transient rise of serum transaminase levels. The relationship of this finding to ampicillin treatment is not clear since to our knowledge elevations of transaminase levels caused by ampicillin have not been reported. In the present material three of the 43 patients receiving nalidixic acid showed a transient elevation of serum transaminase levels. Nalidixic acid is known occasionally to cause mild elevations of transaminase levels. However more serious hepa totoxic effects are apparently rare. Visual disturbances and vertigo are peculiar side-effects which are not uncommon during nalidixic acid treatment, but these side-effects are usually mild and reversible after the discontinuation of the treatment. Phototoxic skin reactions occurred in the present series in two of 43 patients treated with nalidixic acid.

As regards the influence of side-effects on the usefulness of ampicillin and nalidixic acid in the treatment of urinary tract infections the following conclusions may be drawn. The main limitation in the use of ampicillin is the relatively high prevalence of penicillin allergy Other side-effects of ampicillin are less serious, the most common among them being diarrhoea. On the other hand, the side-effects of nalidixic acid have a rather wide spectrum and their frequency is relatively high, in the present series every fourth patient experienced some side-effects.

A commonly accepted and apparently well founded opinion is that nalidixic acid does not belong to the drugs of "first choice" such as sulphonamides and nitrofurantoin, in the treatment of urinary tract infections. However taking into consideration the rather favourable results obtained with nalidixic acid those communities in which E. coli strains do not show high prevalence of primary resistance to this

drug, in our opinion this drug deserves to be regarded as one of the alternatives among the drugs of "second choice in the short-term treatment of urinary tract infections caused by E. coli.

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RETICULUM CELLS AND ERYTHROBLASTS IN THE BONE MARROW OF ANAEMIC PATIENTS

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Abstract. Reticulum cells surrounded by erythroblasts, i.e. erythroblastic islands, were found to be uncommon in bone marrow from haematologically normal persons. Less than 1% of the erythroblasts were connected with reticulum cells. From consecutive series of bone marrow smears examined in routine diagnostic work, 16 patients with an impressive occurrence of erythroblastic islands were selected. Nine of the 16 patients had malignant diseases or diseases associated with inflammatory lesions of various types, and their Hb concentration was low compared to the controls. In the patient group there was also an abnormally large proportion of basophilic erythroblasts within the erythropoietic series. Bone marrow from 5 patients was incubated in autochthonous plasma at 37°C for 1 hour. A reduction of the proportion of erythroblasts connected with reticulum cells was then noted and during the same period there was an increase of the proportion of reticulum cells containing Feulgen-positive cell debris. It is suggested that reticulum cells may engulf and destroy erythroblasts and that this phenomenon occurs frequently in patients with anaemia associated with malignancy and infections. The phagocytosis of erythroblasts may indicate an ineffective erythropoiesis.

In bone marrow smears reticulum cells surrounded by erythroblasts are often found (Fig. 1). Bessis (3) concluded from electron microscopy studies that the erythroid cells obtained iron in the form of ferritin from the reticulum cells. Seki et al. (8) and Awai et al. (1) have suggested that the reticulum cell may phagocytose nuclei extruded from the erythroblasts during their maturation. Thereafter phagocytosed components of DNA might be reutilized by new erythroblasts during their development (7). Thus the "erythroblastic islands" have generally been regarded as a morphologic expression of functions associated with the building up and maturation of erythroblasts. Bessis and Breton-Gorius (4) stated, however that there is no absolute proof that the opposite process does not take place, i.e. the erythroblasts impart material to the reticulum cells.

We have noted that in several bone marrow smears some erythroblasts are apparently situated within reticulum cell (Fig. 2). Their morphology is sometimes deranged, with disruption of the cytoplasm and pyknotic nuclei. In addition the reticulum cells often seem to contain cell debris (Fig. 3). The impression from such smears is that erythroblasts may be phagocytosed and destroyed by the reticulum cells rather than obtain useful building material for their normal development. This is further strengthened by the impression that erythroid islands are often found in patients with anaemia. The purpose of the present work was to find out whether a high proportion of bone marrow erythroblasts in close contact with reticulum cells may be associated with anaemia. In addition the possibility of phagocytosis and destruction of erythroblasts by reticulum cells has been investigated by studies of the bone marrow before and after incubation in vitro.

MATERIAL AND METHODS

Patients. During the daily routine examination of bone marrow smears stained with May-Grünwald-Giemsa, preparations with erythroid islands were collected for about 2 months. Sixteen patients with an impressive occurrence of erythroid islands were recorded in this way. In each patient 1 000 erythroblasts were examined and the proportion of cells in close connection with reticulum cells was calculated. In each patient 100 erythroblasts were morphologically classified and the proportion of basophilic erythroid cells within the erythroblastic series was determined. The Hb concentration was assayed and attempts were made to get as correct information as possible on the ultimate diagnosis (Table I).



Fig. 1 Reticulum cell surrounded by erythroblasts. Erythroblastic island"

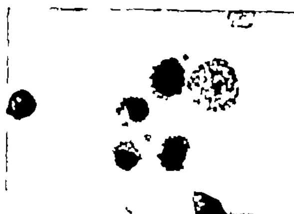


Fig. 2. Four erythroblasts apparently situated within a reticulum cell.

Controls. Nine haematologically normal patients under investigation at our department were examined The control material consisted of patients with angina pectoris admitted for observation and without any signs of myocardial infarction, patients under investigation for suspected endocrine disorders without abnormal findings and patients about to start weight-reducing regimens. Hb concentration, RBC, WBC, differential and platelet counts and ESR were normal in all control patients. The proportion of erythroblasts in connection with reticulum cells was calculated and the proportion of basophilic erythroblasts was determined.

Incubation in vitro of bone marrow Bone marrow 2 ml, was aspirated from the sternum and ejected into 2 ml heparinized autochthonous plasma. Smears were prepared on slides using an artist's brush. The cell suspension was then incubated at 37°C for 1 hour and smears were prepared as described. All preparations were stained with May-Grunwald-Giemsa. In each smear 50–100 reticulum cells were examined (× 1000) and the average number of erythroblasts in connection with a reticulum cell was calculated. The proportion of reticulum cells containing cell debris was determined at the same time In some smears prepared after 1 hour of incubation Feulgen staining was performed according to Bancroft (2) Clusters of Feulgen-positive particles were localized and after staining with May-Grunwald-Giemsa the same focal field was examined.

RESULTS

The proportion of erythroblasts in close connection with reticulum cells or apparently lying within them was significantly higher in the patient group than in the control group (Fig. 4). This was to be expected since the patients were selected with regard to an impressive occurrence of erythroid islands in the bone marrow

The cell composition of the erythropoietic series was found to differ in the two groups. In the patient group with high numbers of erythroid islands the percentage of basophilic erythroblasts was higher than in the control group ($p = 0.025$ Fig. 5) In the patient group with high numbers of erythroid islands anaemia was common and the Hb concentration significantly lower than in the control group (Fig. 6).

Bone marrow from 5 patients with high numbers of erythroid islands was incubated in vitro (Fig. 7). After incubation for 1 hour the number of erythroblasts connected with reticulum cells was reduced in all cases. At the same time there was an increase in the proportion of reticulum cells containing what appeared to be cell debris. The cells often contained large fragments suggestive of damaged nuclei and smaller particles were still more common. Staining with Feulgen and thereafter with May-Grünwald-Giemsa proved that part of this material was DNA



Fig 3 Reticulum cell with cell debris.

Table I. *Clinical data on 16 patients with impressive occurrence of erythroblastic islands in the bone marrow*

	Pat. no.	Sex	Age (y)	Diagnoses	Hb (g/100 ml)
Malignant tumours and/or diseases with inflammatory lesions	1	♀	83	Mammary cancer 1969. Urinary tract infection. Folic acid deficiency	10.1
	2	♂	69	Chronic pyelonephritis, uraemia. Haemorrhagic cystitis	8.1
	3	♂	83	Gastric carcinoma	8.0
	4	♀	3	Enlarged lymph nodes, fever. Toxoplasmosis?	13.1
	5	♂	74	Gout. M-protein in serum	14.1
	6	♂	9	Serous meningitis	12.9
	7	♂	48	Chronic glomerulonephritis. Gastric ulcer. Iron and folic acid deficiency	7.8
	8	♀	71	Pyelonephritis	12.1
	9	♂	56	Serum hepatitis. Venous thrombosis	13.5
Blood diseases	10	♀	85	Pernicious anaemia. Basal cell carcinoma	8.4
	11	♀	55	Pancytopenia of unknown aetiology	11.4
	12	♀	59	Periods of sideropenic anaemia since 4 years. Bleeding never proven	4.7
	13	♂	58	Anaemia of unknown origin. No bleeding or haemolysis	4.0
Other diseases	14	♀	74	Bronchial asthma, osteoporosis. Diabetes	14.2
	15	♀	6	Fanconi syndrome	11.1
	16	♂	41	Alcohol addiction. Epilepsy. Sideropenic anaemia. Bleeding not proven	8.0

DISCUSSION

It is obvious that erythroid islands are not frequently met with in haematologically normal persons. Bone marrow smears are most often obtained in order to find out the cause of anaemia. The haematologist is therefore continually confronted with material from patients with diseases causing anaemia and, more seldom, with marrow from healthy persons. This fact may be one reason why high frequency of erythroid islands as a possible pathological sign may be overlooked. Another reason for neglecting such a finding may be that erythroblasts are generally considered to benefit from the presence of reticulum cells and that normal erythropoiesis is supported by material obtained from them (4, 7).

Our patients with high numbers of erythroid islands constituted a heterogeneous group as regards diagnoses and the phenomenon is therefore by no means specific as a diagnostic sign. However there were several patients with malignant disorders and diseases characterized by inflammatory reactions. A common finding was anaemia and we do not believe that either bleeding or haemolysis was a major cause although this could not be ruled out in all cases. We have recently examined bone marrow from two patients with severe anaemia due to intestinal bleeding from non-malignant lesions and in both cases the proportions of erythroblasts connected to reticulum cells were within a normal range. The findings of large numbers of erythroid islands may therefore possibly be associated with anaemia caused by other mechanisms than loss of mature red cells.

In bone marrow smears rich in erythroid islands some reticulum cells seem to contain whole erythroblasts. In addition damaged cells of uncertain origin are often seen. This gives the impression that reticulum cells may phagocytose

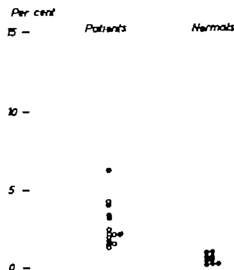


Fig. 4. Proportion of erythroblasts connected with reticulum cells in 16 patients and 9 controls.

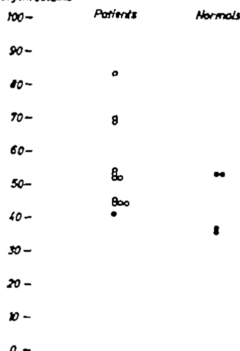


Fig 5 Proportion of basophilic erythroblasts within the erythropoietic series in 16 patients and 9 controls.

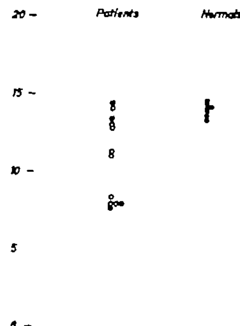


Fig 6 Hb concentration in the patients and controls.

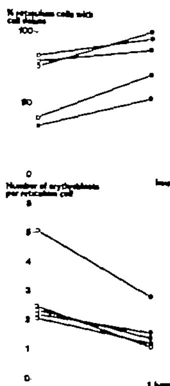


Fig 7 Number of erythroblasts per reticulum cell and proportion of reticulum cells with cell debris before and after incubation of bone marrow for 1 hour at 37°C.

and destroy other cells. The present results obtained with bone marrow incubated in vitro may pport this view Thus, the number of erythro- sts in contact with reticulum cells diminished th time. A denucleation of the erythroblasts as suggested by Awai et al. (1) would result in such reduction of the erythroblasts. According to Bessis and Breton-Gorius (4) the erythroblasts most closely connected with the reticulum cells are the most immature ones and our observa tions are in line with this statement. The reduc tion of erythroblasts was noted after 1 hour of incubation. For basophilic erythroblasts to develop into more mature cells where denucleation occurs, a much longer period is certainly necessary (6). Therefore we believe that denucleation of the erythroblasts does not account for the reduction of erythroblasts in connection with the reticulum cells.

Bessis and Breton-Gorius (4) have pointed out that the reticulum cells extend pseudopodia which occasionally surround the erythroblasts. This is a typical behaviour of phagocytosing cells and, as mentioned we have often noted erythroblasts apparently completely phagocytosed by reticulum

cells, and phagocytosis may well explain the reduction of erythroblasts in connection with reticulum cells during incubation of the bone marrow. An increase in the proportion of reticulum cells containing cell debris was also noted during the incubation. Ingestion of erythroblasts into the reticulum cells and destruction of the ingested material is in agreement with this finding. The presence of Feulgen-positive fragments in the reticulum cells may likewise be compatible with the hypothesis of phagocytosis of erythroid cells. There is, however, no proof that the nuclear fragments did not originate from other cells or from phagocytosed extruded erythroblast nuclei.

From the experiments with incubation of bone marrow several results may thus support the view that erythroblasts are phagocytosed by reticulum cells in vitro. In bone marrow analysed immediately after aspiration there was a predominance of immature basophilic erythroblasts in the patients with anaemia and erythroid islands. If there was an in vivo destruction of erythroblasts, the replenishment of the erythroid pool would probably result in a predominance of young erythroblasts. Whether the destroyed erythroblasts are compensated for through flow from a stem cell compartment or through proliferation of the most immature erythroid cells, the result would be a "shift to the left" within the erythroid series. The high percentage of basophilic erythroblasts found in the patient group is therefore compatible with the hypothesis of an increased destruction of erythroblasts.

The patients with impressive numbers of erythroid islands in the bone marrow were, as mentioned, *generally anaemic. This raises the* question whether phagocytosis of erythroblasts was contributory cause of anaemia in these patients. Severe anaemia has previously been found in association with in vivo phagocytosis of erythroblasts by reticulum cells and histiocytes. Varadi et al. (9) described this in children and termed the condition haemophagocytic reticulosis. A similar syndrome has been described in adults as histiocytic medullary reticulosis, in which Greenberg et al. (5) found phagocytosis of erythroblasts into reticulum cells. The diagnoses mentioned were not justified in our cases but it cannot be ruled out that the pathogenesis of anaemia might be similar. It would seem probable that a high rate of phagocytosis of erythroblasts would be associated with anaemia due to an ineffective erythropoiesis. Further investigations of the problems set forth are in progress.

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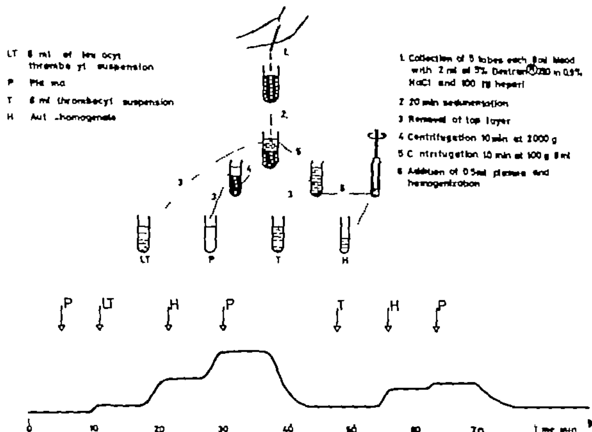


Fig 1 The assay procedure used for the CAL-LE test. At the bottom of the figure a thermogram of a positive test shown. The arrows indicate the times of changing een plasma and the cell suspensions or adding of genate. The baseline at time 0 is obtained with ne. A new baseline is established about 10 min after the introduction of plasma when it reaches and fills the measuring cell. The plateau corresponding to the HP of the LT suspension is obtained after similar interval. The following events are produced in the same way. For each plateau the HP is measured as the difference from the plasma baseline. In a test performed on blood from normal subject the plateau after the first addition of homogenate would be approximately the same as before.

in detail earlier (12, 14), as well as the method of preparation of the leucocyte-thrombocyte (LT) and the thrombocyte (T) suspension (10).

The methodology is summarized in Fig. 1. The essential steps are outlined in the following: 16 ml of LT suspension was obtained from heparin-dextran blood after spontaneous sedimentation. Half of this was subjected to light centrifugation (100 g) for 10 min which forced most of the leucocytes to sediment. The supernatant was the T suspension, whereas the sediment was homogenized in 0.5 ml of the subject's own plasma with a motor-driven Potter Elvehjem homogenizer immediately before use. This is called the autohomogenate (H). To measure HP the patient's plasma was first introduced into the calorimeter followed by the 8 ml of LT suspension. When 4 ml remained in the test-tube 100 µl of autohomogenate was added with a Linderstrøm-Lang pipette and mixing was ensured by gently blowing air through the pipette. The fluid flow into the calorimeter was not interrupted during this step. After the last of the LT suspension containing homogenate had entered the calorimeter a wash with the patient's plasma was made until the basal HP level was approached. This was followed by the T suspension, and addition of homogenate was done in the same way to this suspension. A partly reversed procedure with T suspension before LT suspension and no plasma wash in between has also been used. But the order first described was preferred as it eliminates the risk of transferring material from the homogenate to the first part of the LT suspension.

The numbers of leucocytes and thrombocytes are counted in the two suspensions and the HP/10^9 cells was calculated for each kind of cells with and without homogenate added as described earlier (10). The excess HP from the leucocytes after addition of homogenate was then calculated and expressed as percent increase over each subject's basal value, i.e., percent stimulation. Previously a coefficient of variation of ±10.7% has been found for the determination of leucocyte HP (10).

LE cells. A modification of Snapper and Nathan method (24) was used as described by Möllerberg (15). This has proved reliable during 10 years' use in our

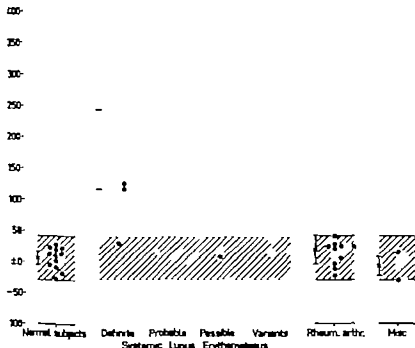


Fig. 2. Results obtained with the CAL-LE test in normal subjects and patients. Only the highest value from each subject is indicated. The crosses and vertical bars to the left of the columns illustrate the mean value ±2 S.E.M. for the groups that contain more than 5 subjects. The shaded area indicates the reference range calculated from the mean value ±2 S.D. of the normal subjects.

laboratory. The results were graded from 0 to 3+ depending upon the number of LE cells in each preparation.

ANF was determined by the immunofluorescence technique (26). The decomplemented sera to be tested were diluted 1/10. Tissue sections of human thyroid gland, rat stomach and rat kidney were covered with serum dilution on microscope slide and incubated for 30 min at room temperature. After rinsing, the slides were reincubated with fluorescein-isothiocyanate (FITC) conjugated sheep antihuman immunoglobulin (1). The preparations were examined in Leitz Orthoplan microscope with incident illumination using standard filter equipment for FITC. Sera with demonstrable antibodies in the first dilution were titrated in the dilution series 1/25, 1/100, 1/400 and 1/1 600.

Other methods. Immunoglobulins have been determined by radial diffusion technique (Mancini) using commercially prepared agar-diffusion plates (Behringwerke, Marburg, West Germany). Other laboratory procedures were those in routine use during the period of investigation.

Normal subjects and patients. The normal subjects examined to obtain reference values were members of the laboratory staff. One value (53% stimulation) was excluded as the subject had a case of SLE in the family.

The patient material consists of a selection among hospitalized subjects and subjects attending the Outpatient Department of St Erik Hospital. They had an established or suspected SLE diagnosis, rheumatoid arthritis (RA) or in some cases, other known collagen diseases.

The classification into diagnostic groups was made according to Dubois (5). Thus all patients with unequivocal LE cells on two or more occasions were regarded as having definite SLE with the exception of patients with RA without other manifestations of SLE. No patients with lupoid hepatitis or drug-associated SLE were encountered during the study. Patients with negative LE cell tests but with clinical manifestations were classified into the groups definite, probable and possible SLE when certain major and/or minor criteria were fulfilled (5).

In the present study an ANF titre was defined as high when positive in serum diluted 1/100 or more and anemia as present when the Hb value was less than 11.0 g 100 ml in the absence of iron deficiency. The symptoms and manifestations recorded in the Tables were taken from the entire length of the current episode of disease, whereas the laboratory values in the Tables are results of analyses performed within the same week as the CAL-LE test. The treatment listed was that being given at the time of the test. Only continuous treatment with corticosteroids, antimalaria drugs and immunosuppressive drugs was recorded.

RESULTS

In the lower part of Fig. 1 a schematic thermogram of a positive CAL-LE test is shown. In general it was found that the test could be carried

Table I *Clinical data and laboratory findings in patients with definite SLE*

Case no.	Sex	Age (y)	Date	CAL-LE test % stimulation	LE cells	Serum ANF titre	ESR (mm/h)	Immunoglobulins (mg/100 ml serum) IgA	IgM	IgG	Major criteria
			Reference values	−30–+40	0	0	2 20	30–300	40–250	700–1 400	
1	♀	26	70 06 24	424	+ + +	≥ 1/400	104	220	95	740	Butterfly rash, polyarthritis, nephritis
2	♀	26	[illegible] 10 17	347	+ +	1/400	12	532	227	3 180	Light-sensitive dermatitis, polyarthritis, typical histopathology on skin biopsy
3	♀	53	73 02 23	250	+	1/100		235	532	1 440	Light-sensitive dermatitis, polyarthritis, pericarditis
4	♀	82	71 12 01	89	+	≥ 1/1 600	51	220	148	1 440	Polyarthritis
4b			72 06 19	221	+ + +		77				Polyarthritis, pleuritis, pneumonitis
5	♀	56	71 10 18	166	+ +	≥ 1/400	45	236	40	1 260	Polyarthritis, pneumonitis
6	♀	40	72 10 31	156	+	1/100	90	382	235	3 000	Light-sensitive dermatitis, polyarthritis, mental depression
7a	♀	26	72 02 29	129	+	1/100	25				Light-sensitive dermatitis, polyarthritis, nephrotic syndrome
7b			72 07 05	34	(+)	1/10	50	98	210	824	
8	♀	23	72 02 10	125	+ +	1/100	6				Polyarthritis, nephritis, kidney biopsy compatible with lupus nephritis
9	♀	43	72 09 20	119	+ + +	≥ 1/1 600	43	390	65	1 230	Polyarthritis, nephritis, kidney biopsy: compatible with lupus nephritis
10	♀	26	72 09 14	117		1/10	7				Polyarthritis, nephrotic syndrome, positive Wassermann reaction, kidney biopsy compatible with lupus nephritis
11	♀	25	70 06 25	8							Polyarthritis, nephritis, hemolytic anemia, kidney biopsy compatible with lupus nephritis
11b			70 09 29	117	+	1/100	129				
11			70 10 23	21	0	1/25	67				
12	♀	46	72 04 26	74	+ +	1/400	8				Loss of hair polyarthritis, thrombocytopenia
13a	♀	23	72 10 06		+ +	1/400	15	124	430	620	Polyarthritis, nephritis, kidney biopsy: compatible with lupus nephritis
13b			73 02 22	32	0	1/10	17	244	96	880	
14a	♀	70	72 02 02	−4	0	1/25	87	148	252	1 620	Polyarthritis
14b			72 09 27	0		1/100	72	229	210	2 244	Polyarthritis, pericarditis
14c			72 10 27	−6			93				
14d			73 01 04	29			73				

out with either of the flow microcalorimetric set-ups as described previously (10 12). Irrespective of whether gold or teflon tubing was used in the flow system it was found necessary to clean the instrument scrupulously with sodium hydroxide or a strong detergent solution before the performance of a test. In one case (no. 13) it was not possible on repeated assays to obtain

Minor criteria	Diagnosis	Treatment at time of study
Fever	Definite SLE	Prednisolone 10 mg
	Definite SLE	
Fever	Definite SLE	
Fever, anaemia	Probable SLE	
Fever	Definite SLE	Prednisolone 90 mg
Fever anaemia, splenomegaly	Definite SLE	Prednisolone 15 mg
Anaemia	Definite SLE	
Anaemia	Definite SLE	Prednisolone 45 mg Azathioprine 150 mg Prednisolone 30 mg Azathioprine 150 mg Cyclophosphamide 50 mg
Fever	Definite SLE	Prednylidene 12 mg Chloroquine 150 mg
Anaemia	Definite SLE	
Fever dermatitis	Definite SLE	Prednisolone 20 mg
	Definite SLE	Prednisolone 30 mg Azathioprine 150 mg
		Prednisolone 15 mg Azathioprine 100 mg Prednisolone 45 mg Azathioprine 150 mg
Fever	Definite SLE	Prednisolone 10 mg
Fever	Definite SLE	Prednisolone 60 mg
Fever Fever diarrhoea Fever	Definite SLE	Prednisolone 20 mg Azathioprine 100 mg Prednisolone 10 mg Prednisolone 30 mg Prednisolone 30 mg Prednisolone 20 mg Chloroquine 150 mg

a proper basal reading of the HP of the leucocytes during a period of high disease activity The spontaneous HP was very high and the thermogram irregular

Table II. *Correlation between results from patients with definite SLE when the stimulation obtained with the CAL LE test is compared with the capacity of the patient's serum to produce LE cells*

CAL-LE test % stimulation	LE cells 0	(+)	+	++	+++
<40	3	1			
40–100			1	1	
100–200			3	2	1
200–300			1		1
>300				1	1

The results obtained in normal subjects and various groups of patients are given in Fig. 2. Case 13 described above was not recorded in the figure. Only one of the 14 patients with definite SLE fell within a reference range calculated for the group of normal subjects. In the borderline groups probable" possible" and variants" 4 out of 11 results were within the reference values, whereas only 2 out of 16 patients with RA showed elevated values. In the group of miscellaneous diagnoses no elevated value was found.

Clinical data, certain laboratory findings and current treatment of the SLE patients are recorded in Table I. The patients are arranged in descending order according to the highest value for the leucocyte HP stimulation obtained in each patient. For each patient the results are arranged chronologically There is a correlation between the capacity of the patient's serum to produce LE cells and the degree of stimulation of the HP of the leucocytes upon the addition of autohomogenate (Table II). A similar but less obvious relation seems to exist between the results of the CAL LE test and the titration of ANF These relations are also seen in individual patients such as 4 11 and 13 for whom the three laboratory procedures were performed during different stages of the disease.

From patient 4 the first sample was taken when she clinically exhibited a picture of RA, but the laboratory findings indicated a probable SLE. The second investigation was made 6 months later when the patient had developed pleuritis and pneumonitis and the diagnosis of SLE was established. At this time she had been on corticosteroid treatment for one week, which had depressed her fever but not the factors respon-

Table III *Clinical data and laboratory findings in patients with probable possible and variants of SLE*

Case no.	Sex	Age (yr.)	Date	CAL LE test % stimulation	LE cells	Serum ANF titre	ESR (mm/h)	Immunoglobulins (mg/100 ml serum) IgA	IgM	IgG	Major criteria
Reference values				−30–+40	0	0	2–20	30–300	40–250	700–1 400	
15	♀	56	72 01 28	269	0	1/1 600	72	360	160	2 600	Polyarthritis, vertigo
15b			72 01 31	369							
16	♀	70	72 10 30	125	0	1/1 600	115	580	71	5 480	Polyarthritis, vertigo
17	♂	53	72 07 04	79	0	Neg.	150	382	156	3 100	Polyarthritis, nephritis
18	♀	44	72 10 30	47	0	1/400	81	290	261	1 940	Polyarthritis, false positive Wassermann reaction
19	♀	22	72 02 02	105	0	>1/1 600	9				Nephropathy
20a	♀	19	70 06 03	0							
20b			72 04 24	50	0	Neg.	28				Polyarthritis
21	♀	42	7? 07 05	18	0	Neg.	42	118	160	885	Nephritis, vertigo
22	♀	59	72 12 21	7	0	1/400	44				Polyarthritis
23	♂	66	70 05 05	89	++	1/1 600	78				Polyarthritis
24	♀	56	70 12 13	36	+	1/100	30	220	198	2 000	Polyarthritis
25	♀	56	70 05 05	14	+	Neg.	72				Polyarthritis

sible for the leucocyte HP stimulation and the capacity to produce LE cells.

Case 11 was one of severe SLE with advanced ...dney involvement. The CAL-LE test was per...med three times during a comparatively stable ...ate of the disease. The first, 11 a, was made when the disease was controlled with high doses of prednisolone and immunosuppressive treatment. Later an attempt was made to lower the dosage but at the time of the second test, 11 b the patient showed signs of clinical deterioration and increased proteinuria. After this investigation the dosage was increased considerably with accompanying clinical improvement. The third set of tests, 11 c, was performed one month later during this stage.

Case 13 was also investigated three times (only one is recorded in Table I) during a few weeks hospitalization because of a SLE with mainly kidney involvement. During this time high doses of prednisolone were administered. As mentioned above, no satisfactory basal HP of the leucocytes could be obtained, probably due to a spontaneous stimulation which might lead to increased adhesiveness and aggregation of the leucocytes. The patient responded favourably to the treatment and was discharged from the hospital with a marked kidney function as well as clinical improvement. The second set of tests recorded in Table I was performed at a control visit 4 months later when she was on a maintenance dose of prednisolone.

The ESR apparently follows the activity of the disease in each individual but shows very little correlation with the other parameters studied. The immunoglobulins varied considerably. Increased values of all the three classes have been found, but a simultaneous increase of all three was not noted in any patient. It is notable that in three patients with kidney involvement IgM and IgG were normal.

In the borderline groups (Table III) it is obvious that the four patients referred to the group probable SLE" mainly on account of their clinical findings all showed elevated CAL-LE test results even though no LE cells could be demonstrated. On the other hand in the group of "SLE variants" only two patients out of three had a positive CAL-LE test although LE cells were found in all three. In the group of patients with possible SLE" two CAL-LE test results out of four were elevated. The patients classified as RA (Table IV) differ from those designated as SLE variants only by the

Minor criteria	Diagnosis
Oral mucosal changes, kerato-*conjunctivitis sicca*	Probable SLE
	Probable SLE
Fever dermatitis, anaemia	Probable SLE
	Probable SLE
Proctitis, anaemia	Possible SLE, *nephrotic syndrome*
Fever seborrhoic facial eczema	Possible SLE
Fever, anaemia	Possible SLE, nephritis
Fever	Possible SLE
	SLE variant, RA
	SLE variant, RA
	SLE variant, RA

demonstrability of LE cells in the latter group. The results obtained with the CAL-LE test closely resemble one another (Fig. 2) The variant" group is, however small.

Among the miscellaneous cases (Table V) were included five patients with arthritis without rheumatoid characteristics and two with elevated ESR with no clinical findings. Several of these cases exhibited an increase of the serum ANF titre as well as increased immunoglobulin values. None had a positive CAL-LE test or LE cells.

In some cases with positive CAL LE test a small portion of the LT suspension with added autohomogenate was taken out. The leucocytes were sedimented by centrifugation and smears were made on microscope slides and stained. Leucocytes *resembling* LE cells could not be detected in any of these preparations.

DISCUSSION

Since the discovery in 1957 by several groups, of circulating antibodies against DNA in SLE (3 19 23) most research efforts concerning this syndrome seem to have been directed towards

Table IV *Clinical data and laboratory findings in patients with RA*

Case no.	Sex	Age (y)	Date	CAL-LE test % stimulation	LE cells	Serum ANF titre	ESR (mm/h)	Immunoglobulins (mg/100 ml serum) IgA	IgM	IgG	Major criteria	Minor criteria	Diagnosis
				Reference values									
				−30–+40	0	0	2–20	30–300	40–250	700–1 400			
26	♀	35	72 01 18	142	0	Neg.	12	156	164	980	Polyarthritis		RA
26b			72 04 28	14	0	Neg.	23						RA
27	♀	78	72 02 03	82	0	1/400	71	41	163	1 200	Polyarthritis		RA
28	♀	29	72 02 07	39	0	1/400	40	171	172	1 300	Polyarthritis		RA
29	♀	32	72 05 16	25	0	1/100	24				Polyarthritis	Fever	RA
30	♀	62	72 02 08	23	0	>1/400	94				Polyarthritis		RA
31	♂	72	71 12 20	±0									
31b			72 11 13	22		1/10	36	410	218	1 940	Polyarthritis	Fever anaemia	RA
32	♀	52	72 11 13	22	0	1/25	33	564	163	1 180	Polyarthritis		RA
33	♀	64	72 02 16	22	0	Neg.	20				Polyarthritis		RA
34	♀	36	71 12 21	20		Neg.	44	176	198	1 000	Polyarthritis		RA
35	♀	24	72 02 07	9		1/10	74	250	132	1 580	Polyarthritis		RA
36	♀	38	71 12 20	3	0	1/25	65	136	172	1 370	Polyarthritis		RA
37	♀	18	71 12 29	−5	0	Neg.	122	256	58	3 000	Polyarthritis	Fever	RA
37b			72 01 28	−10		Neg.	48						RA
38	♀	29	72 05 05	−14	0	1/25	42				Polyarthritis	Fever	RA
39	♂	51	72 06 07	−23		Neg.	56	342	114	1 974	Polyarthritis		RA
40	♀	75	72 06 29	−58	0	1/10	94	424	186	2 820	Polyarthritis	Anaemia	RA

Treatment at time of study prednisolone, 15 mg.

Table V *Clinical data and laboratory findings in patients with miscellaneous diseases*

Case no.	Sex	Age (y)	Date	CAL-LE test % stimulation	LE cells	Serum ANF titre	ESR (mm/h)	Immunoglobulins (mg/100 ml serum) IgA	IgM	IgG	Major criteria
				Reference values							
				−30–+40	0	0	2 20	30–300	40–250	700–1 400	
41	♂	17	71 12 23	15		Neg.	53	372	102	1 40	Polyarthritis
42	♀	47	72 05 31	4	0	1/10	74	532	366	3 840	Polyarthritis
43a	♀	21	72 08 16	−13	0	1/1 600	55	106	64	3 560	
43b			72 09 28	0	0	1/1 600	37	154	155	5 320	
44	♀	57	71 12 06	0		1/10	93				False positive Wassermann reaction (polyarthritis)
45	♂	52	72 02 29	−11	0	Neg.	76	350	328	1 460	
46	♂	54	72 09 04	−33	0	1/400	16				Polyarthritis
47	♀	52	72 11 03	−35	(+)	1/10	9	542	307	1 860	Polyarthritis

establishing the presence of these and concomitant serological phenomena. To our knowledge the cellular factors involved in the formation of the LE cell have not been studied in the light of the development made in the understanding of phagocytosis and the biochemical effects taking place during this process. It is now well established that phagocytosis is accompanied by a ulation of glycolysis by the pentose pathway a concomitant increase of oxygen uptake 1 22). It has also been shown that a similar iochemical readjustment can be triggered by antigen-antibody reactions (20 25) without actual phagocytosis taking place. In a previous paper (12) it has been reported that phagocytosis is accompanied by an increased HP from the leucocytes.

The technique presented in this paper was designed with the objective of studying whether the capacity to form LE cells could be regarded as a phagocytosis-like phenomenon and whether this could be used for measuring the degree of SLE activity Care has been taken not to introduce any material foreign to the patient s leucocytes in the test procedure since this might in itself induce a HP response This required the use of a comparatively large volume of blood (40 ml) for each determination. The technique can, however be modified to the exclusive use of patient serum while leucocytes are obtained from an other source. In this case a considerably smaller volume of patient blood is needed.

The slight increase of HP produced by the addition of homogenate to plasma has been disregarded in the present experiments. On an average a 10% apparent stimulation of the HP of the thrombocytes has been noted upon addition of homogenate in normal subjects and patients alike probably caused by enzymatic processes in the homogenate. This small effect will lead to an underestimation of the HP from leucocytes, which explains why in some cases, negative values of the stimulation were obtained.

The agreement between the results obtained with the CAL-LE test and the clinical diagnosis and the degree of activity of the underlying disease is as good as that reported for DNA antibodies (8 16 17) and appears to be somewhat better than results obtained using LE cell demonstration technique (4 6). However the present material is small and does not allow definite conclusions in this respect. Increased titres of ANF could be demonstrated not only in all patients with definite SLE but also in many cases in the RA and miscellaneous" groups and are considered less specific than the other laboratory procedures mentioned (18)

Brandt and Hedberg (2) have reported a somewhat decreased capacity for phagocytosis in granulocytes from SLE patients. This impaired capacity does not seem to have markedly affected the present results. Since LE cells could not be demonstrated in CAL-LE test positive samples after addition of autohomogenate it is, however

Minor criteria	Diagnosis
	Postinfectious arthritis
Anaemia	Arthritis
Asthenia, tiredness	Collagenosis
	Collagenosis
Tiredness	ESR investigation
	Arthritis
	Arthritis

not certain that the increased HP measured is caused by phagocytosis. On the other hand it does not disprove such a mechanism. The increase might be induced by antigen-antibody reactions in plasma. If this is true, it seems reasonable to believe that anti-DNA antibodies take an active part.

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BODY COMPOSITION AND ADIPOSE TISSUE CELLULARITY IN HUMAN OBESITY

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Abstract. Body composition, age at onset of obesity adipose tissue cellularity and metabolic variables hav been determined in 137 obese subjects and controls Weight stability in 34 of the patients could also be judged by repeated examination after about 10 months. Fat cell number correlated strongly ith body fat, while fat cell size increased only ithin the control range of body fat. Above approximately 30 kg body fat, the fat cell enlargement was equally pronounced at all degrees of obesity A cross-sectional analysis of the obese men and women indicated that fat cell size as larger at 30–40 years of age than before and after this age. Cell number was elevated but did not increase with age in the obese group. Groups of younger and older obese women did not differ in adipose tissue cellularity factors. This had the consequence that, in comparisons ith age- and sex-matched control groups, obesity depending primarily on enlarge ment of fat cells seemed to be more frequent in the younger age groups, while in older obese subjects cell number seemed to be more important factor for contribution to obesity Fat cell number correlated positively with body cell mass. The earlier the onset of obesity the more fat cells, particularly when obesity could be traced to infancy Obesity starting at adult age as charac terized by larger fat cells. T types of obesity can be distinguished. One is hypercellular severe obesity with an early onset and an elevated body cell mass. The fat cells may be enlarged or not. The other type of obesity is characterized by fat cell hypertrophy moderately increased body fat and later onset. There is no increase in body cell mass. Plasma insulin was dependent on eight stability Hypertriglyceridemia in obesity as associated ith decreased glucose tolerance, somewhat elevated insulin values, and elevated serum uric acid

In studies on the experimental animal Hirsch and Han (24) have recently revealed factors of importance for the development of adipose tissue The number f fat cells increases at early age and is then fixed in spite of extreme manipulations of caloric intake. During a limited period within the f rst weeks of life, however dietary changes are able to increase the fat cell number (31). Genetic factors seem to modify this time setting (29) and there are considerable differences between species (28). On the basis of a fixed number of fat cells in the adult animal negative or positive caloric balance seems to cause changes in the total fat content only by variations in fat cell size.

In man the same general picture seems to prevail. Fat cell number increases during childhood (16, 25) and is probably constant from the beginning of adult age (45). In adults only enlargement of the fat cells seems to be responsible for the increase in body fat (45) It is so far not known to what extent f t cell number is modified by genetic and dietary means in man.

In human obesity adipose tissue fat cell size (12, 26, 41) and fat cell number (26) are increased. In a small group of obese subjects variations in fat cell size were preliminarily suggested to explain some of the individual differences in body fat (12). The strong dependence of body fat on fat cell number was later shown independently from Hirsch s (25) and this laboratory (15 43). These reports also showed that, in obesity fat cell size varies independently of adipose tissue size (15 25 43). Associations between fat cell size and metabolic characteristics were also established (8, 15 43). By comparisons with rather extensi e randomly selected control materials (8) of both sexes it was possible to suggest that two types of adipose tissue enlargement were distinguishable depending on f t cell hypertrophy or hyperplasia (8 15 43). In comparison with controls, hyperplasia gradually became the portant factor with increasing body f

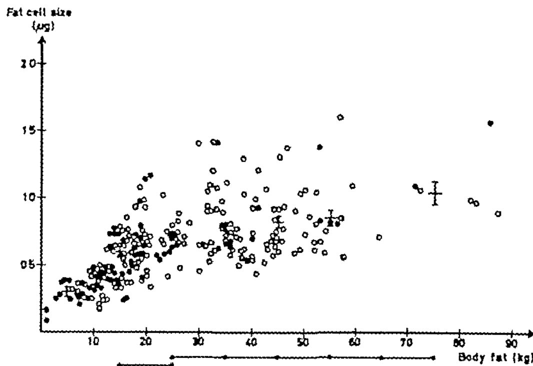


Fig. 1. Dependence of body fat on adipose tissue average fat cell size in 22 young men, 25 young women, 49 randomly selected middle-aged men, 46 randomly selected middle-aged women and 137 obese patients. o = women, • = men. Group mean ± S.E.M. for each 10 kg body fat class, except body fat above 60 kg wher all ers aver aged. Horizontal lines at bottom of figure join mean group values hich are not significantly different on the $p < 0.05$ level by analysis of variance. Data of obese subjects from examination I.

rtrophy was the main reason for moderate ity. The pure hypertrophic type of obesity as not noted by Hirsch and Knittle (25), who examined few patients with moderate obesity. These authors suspected, instead, that the age at onset of obesity might provide a basis for future separation of human obesity into adipose tissue cellularity categories.

Our previous report (15) dealt primarily with adipose tissue fat cell characteristics and their correlations with metabolism, although some preliminary clinical findings in the cellular subgroups were mentioned. In the present work a larger material of obese patients has been analysed, not only as far as adipose tissue is concerned, but also from metabolic and clinical points of view.

MATERIAL AND METHODS

In a circular to outpatient departments and to priv te practitioners in the city of Gothenburg a detailed ex amination of adipose tissue and metabolic variables in obese patients was offered. I this way total of 137 obese patients were made available and examined during 1969–71 (examination I). The results for 37 eight-stable, non-dieting patients have been reported previously (15). Of the 137 patients taking part in examination I, 34 could be reexamined on an average 10 months there after (examination II).

For *examination I* the patients arrived in the laboratory after an overnight fast. They wer asked not to smoke that morning. A venous blood sample was first taken for determinations of plasma insulin, cholesterol, triglycerides and uric acid (17 18, 22, 40). Height and weight were recorded. The mean fat cell size was determined according to Sjöström et al. (44) in percutaneous needle biopsies (23) taken in the gluteal (upper lateral quadrant) bdominal (on line between umbilicus and crista iliaca anterior superior 1/3 from crista) and femoral (on a line between crista iliaca anterior superior and the patella 1/3 from patella) egions. The time of onset of obesity as determined as accurately as possible. Body weight recorded in general medical examinations before school-age (<7 years) and during school-age (7–14 years) provided objec th evidence for time of onset of obesity. It as usually possible to state whether onset of obesity had occurred before school-age, during school-age or during adult age (>20 years). Photographs gav some additional objective evidence, as well as, in some instances, sicknesses during school-years.

In *examination II* tests run in fasting venous blood in examination I ere repeated. Furthermore, peroral

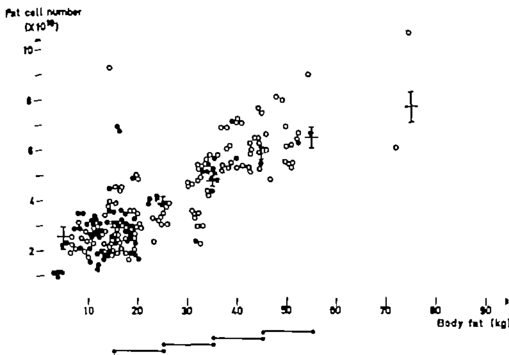


Fig. 2. Dependence of body fat on adipose tissue fat cell number in the same subjects as in Fig. 1. Symbols as in Fig. 1. Data of obese subjects from examination I.

glucose tolerance test was performed with 100 g glucose dissolved in 200 ml water. Venous blood samples were taken at 0, 60 and 90 min for determination of heparin plasma insulin and blood glucose (32). Body composition as also examined by measuring total body water by administration of tritiated water as described by Lindholm (31, 34) and measurements of total body potassium in hole body counter. Body cell mass (BCM) (kg) was calculated from total body potassium (K) (mmole): BCM = K·8.33/1 000 (ref. 4). The equation is based on the assumption of potassium-nitrogen ratio of 3 mmole/g and protein content of 25% of the wet eight of the cells. From these data body fat could be estimated as described by Berg and Isaksson (4).

Body fat was thus determined only in that part of the obese group which took part in examination II. In these and other obese patients (altogether 62 subjects) different correlations between determined body fat and anthropometric measurements were examined. The formula $0.18(W/H^2 \cdot 10^4) - 23.2$, here W is body eight (kg) and H height (cm), was found to yield the highest correlation coefficients of those tested and the following regression equations

$y = 0.75 \quad + 15.3, \quad = 0.84$ ($= 62$, all patients)
$y = 0.62x + 22.5 \quad = 0.75$ ($n = 16$, men)
$y = 0.78x + 13.7 \quad = 0.88$ ($= 46$, omen)

here is the determined body f t and y the calculated body fat from anthropometric data. This formula was devised by Edwards and Whyte (19). Since the correlation coefficients were high, and since the regression equations were calculated on group of obese patients which as not significantly different from the rest of the obese material with respect to body weight, age and sex distribution, it was considered adequate to utilize the formula given above (19) for calculation of body fat in the patients taking part in examination I.

The total fat cell number was calculated by dividing body fat by an average fat cell size obtained from the mean fat cell size of the three regions examined.

As controls for the obese patients several previously described groups of subjects were utilized. Obese men bove the age of 40 were compared with randomly selected men ($n = 45$) aged 55 years (8), and obese women above the age of 40 ith group ($= 54$) of randomly selected 50- and 52-year-old omen (8). For comparisons with obese men below the age of 40 tw previously published control materials of medical students ($= 22$) ere pooled (9 45). Young obese women were compared ith control group consisting of 13 medical students, selected to avoid obesity (45), pooled with another 11 healthy young women (mean age 22, range 19–23 years) examined before the use of contraceptive drugs.

As basis for subdivision of obese women into groups of different body cell mass, another material of randomly selected women from the city of Gothenburg could be utilized. These omen were aged 38 ($n = 19$) and 46 years ($n = 39$). Details from this investigation will be reported by Bengtsson and Isaksson (2).

The examinations of body cell mass in randomly

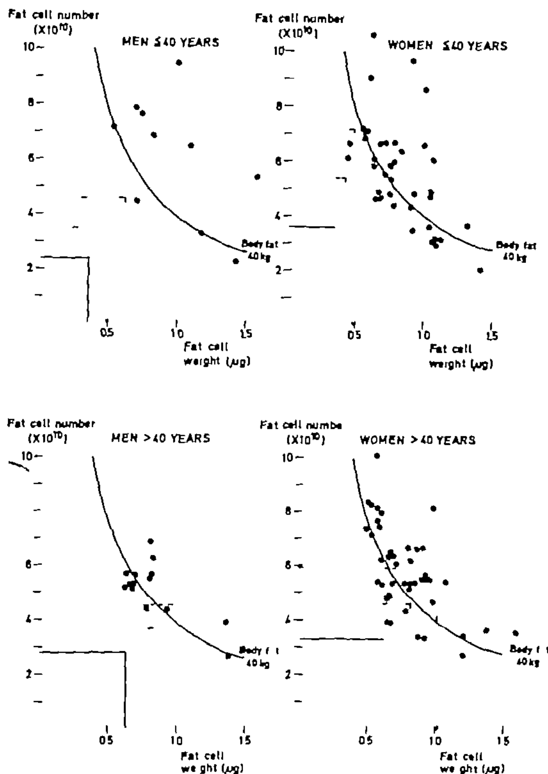


Fig. 3. Subdivision of obese patients into hypercellular and hypertrophic groups in relation to sex and age-matched controls. Rectangles give mean, mean+1 S.D. and mean +2 S.D. for the controls, consisting of young men or women or middle-aged men and women (cf. Fig. 1). Fat cell weight and number corresponding to 40 kg body fat inserted. Data of obese subjects from examination I.

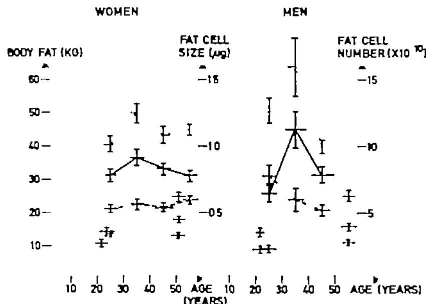


Fig. 4 Dependence on age of body fat ( ), fat cell size (—) and fat cell number ( ) in obese (values connected by lines) and control (values not connected by lines) women and men. Mean ± S.E.M. Data of obese subjects from examination I.

lected women are performed at Medical Department II the Department of Clinical Nutrition and the Institution of Radiophysics, Sahlgren Hospital, Gothenburg. Drs C. Bengtsson and B. Isaksson placed these unpublished data at our disposal.

RESULTS

Fat cell size and number in relation to body fat

Fig. 1 shows the dependence of body fat on fat cell size in the controls and in the obese patients. As described previously (8 15), fat cell size increases within the lower range of body fat, while there seems to be no further increase in fat cell size over approximately 30 kg body fat. Fat cell number on the other hand, (Fig. 2) increases successively over the whole range of body fat.

Contribution of fat cell hypertrophy and hyperplasia to the enlargement of adipose tissue

Adipose tissue cellularity is dependent on age and sex. Subjects in their twenties thus have smaller fat cells than in their fifties, and women have more fat cells than men (45). When comparing the obese patients with the controls, it was therefore considered necessary to analyse men and women, and younger and older patients, separately. Men and women below and above 40 years of age were therefore treated as four separate groups and compared with corresponding controls. This is shown graphically in Fig. 3

In younger obese women the hypertrophy factor seemed to dominate. In women above 40 years of age, on the other hand, the hyperplasia factor seemed to be more prominent and this was particularly pronounced in the severely obese women. The male material was smaller but in general the same characteristics as for obese women seemed to prevail. It should be noted, however that these differences between the contribution of the two cellular factors to obesity are mostly due to the differences between the control materials, because when compared with each other the younger and older obese women showed no difference either in fat cell size or number

Variation of fat cell size with age of control and obese subject

The influence of age on body fat and fat cell size and number was further analysed in different age groups of the obese men and women (Fig. 4), corresponding values for controls are included for comparison. In obese women and men body fat showed a peak value between 30 and 40 years of age. This was due to a larger fat cell size at this age, while fat cell number was constant in different female age groups and showed a lower value in the oldest than in the youngest male groups.

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Table I. *Correlations between adipose tissue cellularity versus body cell mass and age of onset in severely obese subjects (data from examination II)*

x	y	Regression eq.	n		p
Fat cell number (10^{6})	Body cell mass (kg)	$y=2x+16$	34	0.48	<0.005
Fat cell weight (μg)	Body cell mass (kg)	$y=-7x+35$	34	−0.17	n.s.
Fat cell number (10^{6})	Age of onset (y.)	$y=-3x+42$	34	−0.32	<0.10
Fat cell number (10^{6})	Age of onset (y.) (<15 y.)	$y=-1.5x+16$	12	−0.62	<0.05
Fat cell weight (μg)	Age of onset (y.)	$y=26x+2$	34	0.27	<0.10

Correlations between adipose tissue fat cell size and number and other investigated variables

Table I shows that body cell mass and fat cell number correlated positively with a rather high significance while fat cell size was not associated with body cell mass. When the statistical correlation between age of onset and adipose tissue cellularity was investigated, it was found that there was a trend to negative correlation with number of fat cells and a trend to positive correlation with fat cell size. The plot of these data suggested a non-linear association, with a lack of correlation between fat cell number and age of t in adults. Accordingly the correlation be these two variables was fully significant obesity had begun before the age of 15 out of the 12 patients with an onset of obesity before the age of 15 had probably been obese already during infancy. Furthermore, the correlation between age at onset and fat cell size was suspected not to be linear either and therefore all subjects with a debut of obesity before the age of 15 were compared with those whose obesity started after adult age (>20 years) (Table II). The adult onset obesity group had larger and fewer fat cells.

Comparisons between different groups of obese patients based on body cell mass

Because of the rather strong positive correlation between fat cell number and body cell mass (Table I) a division of the obese subjects on the basis of body cell mass was performed. This was done only for women as the number of patients was sufficient for such a subdivision. The body cell mass of the control women provided a basis for this grouping on the following lines.

The body cell mass for the randomly selected control women was 21±3 kg and 22±4 kg (mean ±S.D.) for the 38- and 46-year-old women respectively (2). There was thus no significant difference between these age groups, and therefore they were pooled into one group. The mean +1 S.D. of this group ($n=58$) was then 25 kg, and the mean +2 S.D. 28 kg. In order to get well separated groups, obese women with body cell mass below 25 kg were placed in one group, and all with a body cell mass above 28 kg in another. These two groups are compared in Table III. Patients with high body cell mass were heavier and showed a trend to be fatter. This was due to a greater number of fat cells in the group with elevated body cell mass, while fat cell size was not significantly different between the two groups. No metabolic differences could be demonstrated, but the age of onset was considerably lower in the group of obese patients with high body cell mass.

Metabolic associations

The previously described association between fasting plasma insulin and average fat cell size of the three examined sites (femoral, gluteal and abdominal regions) in obese patients (15) was not found in the present work. Since several patients in the present study, in contrast to the previous report, were dieting and not weight-stable an analysis was made of the influence of this factor. Comparisons between examinations I and II made it possible to analyse to what extent weight changes influenced plasma insulin. Weight changes exceeding 2 kg in body weight were arbitrarily taken as borderlines for weight-increasing and weight-decreasing groups respectively. Subjects with a body weight within ±. kg in comparison with examination I were

weight-stable. As seen in Table IV the weight gaining group had higher insulin values than weight-constant or weight-decreasing groups, although the insulin values of the latter group were still significantly higher than those of middle-aged control women (5 ± 1 µU/ml, fasting value) (8).

When only weight-stable patients were investigated, the correlation between fat cell size and plasma insulin was again found although with a weak degree of significance (= 0.49 $p < 0.05$).

Plasma lipids did not follow any of the subdivisions of the obese group. Therefore this aspect was analysed separately by dividing the obese women according to the plasma triglyceride of the middle-aged control material in an analogous way to the di ision according to body cell mass (see above). The mean +1 S.D (1.2 mM) (8) and mean +2 S.D (1 7 mM) gave the upper and lower borderlines, respectively for normotriglyceridemic and hypertriglyceridemic groups of obese women. These results are listed in Table V Cholesterol, blood glucose and uric acid values were significantly higher in the hypertriglyceridemic group. In comparison with middle-aged control women (8) body fat, fat cell number and fat cell size were elevated in both groups, but fasting blood glucose and plasma insulin were elevated only in the hypertriglyceridemic obese group.

Table II. *Data of obese subjects from examination II divided according to time f onset of obesity (means ±S.E.M.)*

Onset of obesity		Age (y)	Fat cell size (µg)	Fat cell number (10^{10})
In childhood (<15 y)	12	43 ± 5	0 66 ± 0 03	6.9 ± 0 6
As adult (>20 y.)	22	49 ± 3	0.78 ± 0 03	5.7 ± 0.2
p		n.s.	<0 001	<0 05

DISCUSSION

Relationships between adipose tissue cellular factors, body fat and age

Body fat was strongly dependent on fat cell number while this was the case for fat cell size only in the control region of body fat. Above approximately 30 kg body fat the size of the hypertrophied fat cells seemed not to increase further This is in accordance with previous reports from this laboratory (8, 15). Similar results ha e also recently been reported by Lisch et al. (35) and by Gries et al. (21) and, within the obese region of body fat, by Hirsch and Knittle (25).

Fat cell size in the present work was, on an average, somewhat lower than in previous study (15) in which obese patients were, however pre selected so as not to include dieting or diabetic subjects.

Taken together with the data vailable from studies in children and previously reported data from adults, the results from the present study give the following summary of the cross-sectional information on adipose tissue cellularity in non-obese and obese humans in relation to age. In non-obese children a fast increase of fat cell size and number seems to occur during infancy and then a slower increase of both these variables up to adult age (16, 25 30). Middle-aged non-obese adults have larger fat cells than young persons, while fat cell number is not different (45). In obesity the following information is available. Obesity in infancy is apparently associated with a still faster rate of fat cell multiplication than in non-obese subjects (16, 30). In obese patients of adult age there is no evidence of a further increase of fat cell number however Fat cell size also increases above normal in obese children. In obese adult subjects fat cell size seems to increase up to 30–40 years of age.

However this picture is obtained from cross-sectional and not longitudinal data. Furthermore, most materials of patients and controls reported so far hav not been randomly selected. This has the consequence that different types of selective mechanisms might have influenced the results. In the present obese group there was no increase in fat cell number with age, a finding giving no support for a continuing fat cell multiplication in adult human obesity Such a multiplication seems to occur in the genetic obesity of the Zuc ker rat (29). However a definite evaluation of this and similar problems must await longitudinal studies.

The relation between body f t and fat cell size (Fig 1) suggests that adipocyte enlargement ceases at a certain fat cell size. This might mean that regulatory factors prevent fat cells from

Table III. *Data of obese women from examination II divided according to body cell mass (means ± S.E.M.)*

Body cell mass	n	Age (y.)	Height (cm)	Weight (kg)	Body fat (kg)	Average fat cell size (μg)	Fat cell number (10¹⁰)	Glucose (mg ..) 0	60'	90
Elevated (mean + 2 S.D. of controls)	8	49±5	164±2	108±7	48±3	0.78±0.05	6.6±0.3	89±8	160±22	–
Not elevated (< mean 1 S.D. of controls)	6	54±7	162±2	89±6	39±4	0.76±0.05	5.2±0.5	87±8	143±26	146±
p		n.s.	n.s.	< 0.05	< 0.10	n.s.	< 0.05	n.s.	n.	n.s.

Between examinations I and II.

creasing above a given level. When these regulatory factors are disturbed, fat cells increase enormously in size as has been shown in the rat with lesions in the ventromedial hypothalamic nucleus (24). The normal regulatory mechanism of fat cell size seems to have a setting dependent on age. In obesity this setting seems to be abnormally high although there are individual variations.

Incidence of hypertrophic and hyperplastic obesity

differences between the incidence of hyper and hypertrophic obesity in different age groups of the present material were due mainly to differences between the control groups (Fig. 3). The obese subjects in the younger age groups were therefore found to be of a hypertrophic type of obesity when compared with their age and sex-matched controls. Likewise older control subjects had larger fat cells, and therefore the hypertrophy factor was less pronounced in older obese patients, giving more hyperplastic obese subjects in this age group.

Subgrouping of obesity

The results of Tables I–III together with previous investigations (8, 15) indicate that the obesity syndrome in man can be divided into at least two subgroups.

There is one hypercellular type of obesity characterized by an early onset and an elevated body cell mass. Body fat is severely increased due to the large number of fat cells. The fat cells may be enlarged or not.

There is also probably a hypertrophic type of obesity which is characterized by a moderate increase in body fat due to enlarged fat cells. This type has a later onset and there is no increase in body cell mass or fat cell number. The characteristics of such a group are analogous to those of middle-aged controls in comparison with young controls, viz. an increase of body fat at higher age depending on fat cell size enlargement, but not elevation of fat cell number or body cell mass. Such obesity has also been induced experimentally in man (42). In Table II both fat cell size and number in adult onset obesity are somewhat higher than in randomly selected controls (8). However the obese patients of the present study were preselected as being severely obese, probably resulting in a low frequency of purely hypertrophic obese patients. The patients with adult onset obesity in Table II therefore probably constitute a mixed group of obesity.

Some of the observations reported here have been made before. First, the increase not only of body fat but also of body cell mass in obesity has been reported by several authors (20, 36, 37). Forbes (20) suggested that the increase of body cell mass in childhood obesity is found primarily in subjects with early debut of the condition. Among obese adults, juvenile onset obesity seems to be more severe than adult onset obesity (38). These previous reports seem to agree well with the present findings. The association between adipose tissue hypercellularity and juvenile onset of obesity has been suggested previously by Hirsch and Knittle (25) and our laboratory (15). Brook et al. (16) very recently reported analogous data in a more extensive study. The finding of a large

Insulin (μU/ml)			Triglycerides (mM)	Cholesterol (mg %)	Uric acid (mg %)	Weight change[a] (kg)	Age at onset (y.)	Body cell mass (kg)
0′	60	90′						
21±4	101±19	141±36	1.64±0.18	234±9	5.6±0.4	1.4±2.0	10±4	31±1
11±5	76±14	140±56	1.20±0.21	238±16	5.1±1.0	−.1±2.3	27±7	24±1
	s.	n.s.	s.	n.s.	n.s.	n.s.	<0.01	<0.001

increase in fat cell number with juvenile onset in obese subjects in the present work thus agrees with previous reports, and also extends the information to include the increase in body cell mass in these subjects.

Na… (39) has provided evidence suggesting that obesity is characterized not only by an increased cellularity in adipose tissue but also in other tissues of quantitative importance for the total cell mass of the body. It seems quite possible that nutrition and other factors during infancy and childhood are able to trigger hyper

Table IV. *Data of obese subjects from examination II who had increasing, constant or decreasing body weight between the two examinations (means ± S.E.M.)*

Weight		Body cell mass (kg)	Body fat (kg)	Average fat cell size (μg)	Fat cell number (× 10[10])	Fasting insulin (μU/ml)	Time between examinations I and II (mo.)
Increasing between examinations I and II (> 2 kg increase)	11	28±1	47±3	0.72±0.04	6.6±0.5	19±2	10±1
Constant (±2 kg change)	12	28±1	41±2	0.73±0.03	5.7±0.3	13±2	10±1
Decreasing (> 2 kg decrease)	11	26±1	44±3	0.79±0.06	5.8±0.4	11±2	11±1
p (first vs. third line)		s.	n.s.	n.s.	n.s.	<0.01	n.s.

Table V. *Data of obese women from examination I divided according to plasma triglyceride concentration (means ± S.E.M.)*

Plasma triglyceride		Age (y.)	Body fat (kg)	Average fat cell size (μg)	Fat cell number (× 10[10])	Fasting blood glucose (mg %)	Fasting plasma insulin (μU/ml)	Uric acid (mg %)	Chol-esterol (mM)	Trigly-ceride (mg %)
Elevated (> mean + 2 S.D. of controls)	20	43±3	45±3	0.72±0.03	6.3±0.3	94±11	11±2	5.7±0.5	269±17	2.6±0.4
Not elevated (< mean + 1 S.D. of controls)	17	43±3	4 ±3	0.82±0.05	5.3±0.4	69±3	8±2	4.5±0.3	21 ±10	0.9±0.1
p		s.		n.s.	n.s.	0.05	n.s.	0.05	<0.01	<0.001

plasia of several tissues, including adipose tissue (49), producing a syndrome of general cellular hyperplasia.

Metabolic factors

In contrast to a previous report (15) plasma insulin concentration did not correlate significantly with fat cell size in the whole material of obese subjects. However there was a significant correlation in the weight-stable patients. It has been pointed out previously that this association requires a non-restricted diet, but probably a restricted physical activity (14). Diabetes mellitus *prevents the correlation* (13). This correlation seems more reliable in men with no or slight obesity (3 9 11) and has recently been found also *in other laboratories* (46 47 48).

Elevated plasma triglycerides were not associated with any of the body composition or adipose tissue cellularity variables. As reported before hypertriglyceridemia was associated with decreased glucose tolerance, elevated plasma insulin and uric acid values (1 5 6, 7 27). In hypertriglyceridemic patients, selected primarily according to plasma triglyceride values, fat cells were found to be enlarged but normal in number The subdivision according to triglyceride es in the present group of severely obese subs gave no difference in fat cell size between the groups, both having abnormally large fat cells. In contrast to the previously reported hypertriglyceridemic patients (10) both groups of obese patients in the present work also had more fat cells than controls, and this may explain why fat cell size did not follow plasma triglycerides.

Addendum. After this work was *submitted for publication* report has appeared which deals with the same problem (Salans, L. B Cushman, S. W & Weisman, R. E., Studies on human adipose tissue. Adipose cell size and number in nonobese and obese patients. J clin. Invest. 52: 929 1973). The eport by Salans et al. confirms the presence of a hyperplastic and a hypertrophic form *of obesity* (15), the *higher* dependence of body fat on fat cell size than on fat cell number in nonobese subjects (8), and the higher dependence of body *fat* on *fat* cell number than on fat cell size in obesity (15). Furthermore these investigators demonstrate, in agreement with the results reported in the present paper that the hypercellularity of adipose tissue in obesity is dependent on an *early* ge of onset, as well as on increased lean body mass in obese subjects with hypercellular adipose tissue.

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THE EFFECTS OF EARLY MALNUTRITION IN MAN ON BODY COMPOSITION AND ADIPOSE TISSUE CELLULARITY AT ADULT AGE

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Abstract. Ten adult men, who as newborns suffered from pyloric stenosis with severe malnutrition, were probably shorter and showed tendency to smaller body cell mass than controls. Body fat and fat cell size tended to increase with age in controls and pyloric stenosis subjects. *Fat cell number was similar to that of the controls. It was suggested that malnutrition in the earliest period after birth exerts an influence on cell multiplication, but the effect on adipose tissue cellularity is compensated for during recovery from starvation.*

The newborn period of life is characterized by a rapid tissue growth. Studies by Enesco and Le blond (8) and Winick and Noble (20) indicate that this growth is due to an initial phase of increase in the number of cells of the tissues, followed by an increase in number as well as cell size. Finally cell multiplication ceases and the tissue grows by enlargement of the cells. Each tissue seems to have its own period for this development. Nutritional and other factors appear to have pronounced and irreversible effects during the period of hyperplastic growth (20).

The development of tissues such as brain, muscle and liver in terms of cellular growth has been studied in some detail. The influence of nutritional factors on this development is reviewed in recent reports by Cheek and Hill (7) and Winick (19). Only recently however has information been available on the influence of the nutritional state on the development of adipose tissue. Hirsch and Han (10) demonstrated in the rat that the phase of hyperplastic growth of adipose tissue appeared to comprise the first 12–15 weeks of life. Changes in the nutritional level during the first few weeks after birth modified the number of fat cells (12), but after this period the most extreme nutritional variation had apparently no effect on fat cell number and affected only the size of the fat cells (10).

As yet, information on these important questions is lacking in man. Such knowledge may be of considerable importance because the number of fat cells seems to be an important factor for determining the amount of body fat at adult age and thus the tendency to develop obesity (3 5 11) In this report body composition and adipose tissue cellularity were studied in adult men who in early infancy had suffered caloric under-nutrition for several months due to severe hypertrophic pyloric stenosis. They were treated medically and after recovery their caloric intake was normalized. Their opportunity for food intake was then the same as for ordinary Swedish children.

MATERIAL

Ten men, born in 1923–40, who suffered from pyloric stenosis (PS) in the newborn period, were examined. The ailment started 0–5 days after birth and had duration of 11 18 weeks (14 ± 1 mean ± S.E.M.). During this time they ere treated with spasmolytica and given numerous small feedings by spoon, and in some cases subcutaneous salt solution with glucose. Because of the profuse vomiting their caloric intake was deficient until recovery These children decreased in weight to minimum of 40–60% (50 ± 2, mean ± S.E.M.) of the ideal weight for their age At the time of the examination the PS subjects were 40.2 ± 2.6 (mean ± S.E.M.) years of age.

The PS subjects were compared with controls, consisting of 22 healthy male medical students 23 ± 1 years of age (mean ± S.E.M.) (4, 17), and with 45 men aged 55 years, selected at random in the same city (Gothenburg) as the men with PS came from (3).

Table I. *Results of determination in men with pyloric stenosis after birth*

Subject no.	Age (y.)	Height (cm)	Body weight (kg)	Body cell mass (kg)	Body fat (kg)	Fat cell weight (μg) Hypogastric	Gluteal	Average
1	46	176	75	29	13	0.44	0.35	0.40
2	43	169	73	33	5	0.38	0.21	0.30
3	37	174	86	37	16	0.38	0.47	0.43
4	31	175	76	30	15	0.37	0.55	0.46
5	39	179	76	36	10	0.27	0.49	0.38
6	38	179	75	29	15	0.44	0.47	0.46
7	45	176	90	32	29	0.35	0.43	0.39
8	38	174	63	32	6	0.50	0.58	0.54
9	47	167	65	26	16	0.53	0.54	0.54
10	38	167	56	27	5	0.29	0.41	0.35
Means ± S.E.M.	40±2	174±1	74±3	31±1	13±2	0.40±0.03	0.45±0.03	0.41±0.02

METHODS

Body weight and height were measured. Body cell mass was calculated from body potassium as described by Moore et al. (15). Body potassium was determined with a whole body counter detecting naturally occurring ⁴⁰K (18). Total body water was measured by administration of tritiated water (13, 14). When body weight, height, body cell mass and total body water are known, body fat can be calculated as described by Berg and Isaksson (1).

Fat cell size was determined in percutaneous needle (9) obtained from the subcutaneous adipose of the hypogastric region on the midpoint of from the costa iliaca superior anterior and the umbilicus, and in the upper lateral quadrant of the gluteal region. In young men the size of the fat cells is similar in these two regions and, furthermore, it is not different from that of two other major subcutaneous adipose tissue regions, viz. the femoral and epigastric region (17). The samples of adipose tissue obtained were briefly fixed in formalin and the fat cell diameters determined by microscopic method (16). The average fat cell weights of both regions were calculated, and body fat was divided by this value in order to obtain an estimate of total fat cell number of the body.

Statistical methods utilized were analysis of variation and correlational regression analysis.

RESULTS

Table I shows the detailed results of the investigations in the PS subjects, and Table II shows the comparisons with controls. The young controls were taller and had higher body cell mass than the PS subjects and the older controls. Body weight was not significantly different between the groups. Body fat of the PS subjects did not differ from that of any of the control groups. Fat cell weight did not differ between PS subjects and younger controls but the former group had smaller fat cells than older controls. Fat cell number did not differ significantly.

Regression analyses showed a comparably high although not significant correlation coefficient for body cell mass vs. weight decrease ($r \sim 0.50$) in the PS subjects. In a larger material the correlation between adult heights vs. duration of inanition was fully significant (2). There were no significant correlations between adult height, body weight, body cell mass, birth weight, duration of malnutrition or minimum weight during malnutrition on the one hand and adipose tissue data on the other.

DISCUSSION

Entirely comparable controls as far as age is concerned were not available. Controls of either lower or higher age than the subjects with PS were utilized for comparisons. The fact that the PS subjects were shorter than the younger controls suggests that the subsample examined in the present investigation was indeed abnormally short, as described previously (2). Like height, body cell mass was also smaller in the PS subjects.

Fat cell number did not differ between the groups. Although not significant in all comparisons, there was a trend for body fat and fat cell size to increase with age, mean values being lowest in the younger controls, somewhat higher in PS patients and highest in the older controls. The increase of body fat with age is known previously. At adult age it is due to an increase

Fat cell number (10^{-9})	Birth weight (g)	Duration of PS (weeks)	Minimum weight (% of ideal for age)
3.2	4 000	18	46
1.8	3 650	11	46
1.7	3 400	13	60
3.3	4 030	12	57
2.6	4 000	11	55
3.2	4 000	17	45
7.4	3 730	15	40
1.1	3 900	15	56
2.9	3 000	12	43
1.3	3 200	11	49
3.1±0.6	3 691±117	14±1	50±2

in fat cell size rather than number both in man (17) and rat (10). The difference in adipose tissue cell size between the groups is thus that expected due to the differences in age.

The results obtained thus imply that with severe malnutrition during the first 3–4 months of life in man height, and possibly body cell mass, are influenced at adult age, but apparently not the size nor the cellularity of adipose tissue.

Hirsch and Han (10) have recently shown that fat cell multiplication apparently can occur only during the first 12–15 weeks of life in Sprague Dawley rats. In man the findings of Brook et al. (6) indicate that f t cell number in non-obese children increases up to the age of bout 15 years. It is, however only during the first few weeks after birth that this fixed f t cell number can be influenced by exogenous means in the rat (12). If similar conditions prevail in man, it should only be possible to influence fat cell multiplication by nutritional factors for a short period immediately after birth. Thus an influence on the fat cell number of adipose tissue of the men in estigated in the present work would have been expected. Such an influence could, however not be traced.

That no change in adipose tissue cellularity was found in the present study may be due to different reasons. 1 Nutritional factors perhaps do not influence adipose tissue cellularity in man as they do in the rat, as shown by Knittle and Hirsch (12). 2. It may only be over-feeding and not under-feeding that exerts an effect on fat cell multiplication in man. 3 A temporary decrease in fat cell number may have been compensated for by a period of more rapid fat cell proliferation when the children in question recovered from the starvation. Acutely starved rats seem to react in this way (10).

The period of malnutrition in the subjects investigated coincided with a normally occurring lively cell division in different tissues. This probably had the consequence of a retardation of cell multiplication in analogy with what happens in the experimental animal (20). It seems a rather attractive hypothesis that adipose tissue might compensate for this retardation, while other cells are in a phase of development where such compensation is no longer possible after recovery from the starvation. This concept may explain the observation of the present study that the PS subjects probably were shorter and showed a tendency to a decreased body cell mass at adult age.

Caution must be exercised, however in the interpretation of the present results before they are accepted as generally alid for the situation of malnutrition in infancy. Babies with PS may well differ from the general population geneti-

Table II. *Results of determinations in men with pyloric stenosis after birth and controls*

		Age (y.)	Height (cm)	Body weight (kg)	Body cell mass (kg)	Body fat (kg)	Fat cell weight (µg) (Glotzel)	Fat cell number (10^{-9})
I. Young controls	22	23±1	182±7	71±6	35±4	9±6	0.36±0.13	2.4±1.1
II. Pyloric stenosis	10	40±5	174±4	74±10	31±4	13±7	0.45±0.11	3.1±1.8
III. Middle-aged controls	45	55	175±6	73±11	31±4	16±5	0.63±0.25	2.8±0.9
I vs. II			$p<0.01$	n.s.	$p<0.05$	n.s.	n.s.	n.s.
I vs. III			$p<0.01$	n.	$p<0.01$	$p<0.01$	$p<0.01$	
II vs. III				n.s.	n.s.	n.s.	$p<0.05$	

cally or otherwise, which would modify the effects of exogenous factors in the form of for example malnutrition.

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DOSAGE, PLASMA CONCENTRATION AND ANTIARRHYTHMIC EFFECT OF PROCAINAMIDE IN SUSTAINED-RELEASE TABLETS

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Abstract. A new sustained-release tablet of procainamide has been found to have mean biological half-life of 6.7 hours as compared with 3.2 hours in ordinary tablet form. The dosage of 1.2 g t.i.d. yielded veraged plasma concentrations of 3.2–5.4 mg/l after 24 hours. Ventricular premature beats were abolished for about 5 hours in most of the 47 patients studied. These findings suggest 1.0 g q.i.d. to be the optimum for an verage patient. Because of great individual differences the determination of procainamide plasma levels in clinical use is recommended.

Procainamide hydrochloride is used extensively in the prevention and treatment of cardiac arrhythmias. This drug is ffective in both supraventricular and ventricular arrhythmias and because of nearly complete absorption can also be taken orally. The narrow therapeutic range limits its usefulness. Acute toxic symptoms follow an overdose or a plasma concentration of over 12 mg/l.

The mean half-time of the elimination of procainamide has quite recently been demonstrated to be approximately only 3 hours (14 19). For most patients a daily oral administration of 50 mg/kg, *divided into 3-hour doses*, is necessary to prevent fluctuations of more than 50% in the plasma concentration (12). This dosage schedule yields plasma concentrations in the optimal therapeutic range of 4–8 mg/l (5 12). In patients with an impaired renal function the excretion and elimination of procainamide is directly related to creatinine clearance (12, 19). In patients with a low cardiac output and an associated decreased renal function the plasma concentration also tends to rise (12). The half-life becomes longer and the dosage interval must be prolonged respectively (19).

The recommended 3-hour dosage schedule (8 12) is practically impossible in long-term treatment. Therefore sustained-release tablets of procainamide might be a aluable aid in the prevention of arrhythmias (17). This kind of tablet has recently been developed (Lääke-Medipolar Research Center, Turku, Finland). The rate of absorption is reduced and so toxic peak concentrations can be avoided in spite of a relatively infrequent and large dosage of procainamide. This study was aimed at testing this hypothetical principle and t ascertaining the longest possible dosage interval with the new pharmaceutical product.

MATERIAL AND METHODS

Patients. The 47 patients in this study were all hospitalized. All 37 male patients were treated in coronary care unit with constant ECG monitoring on cardioscopes. Twenty-seven patients had had an acute myocardial infarction at least some days previously. Other patients were observed mainly because of frequent ventricular premature beats (VPB). No patient with definitely impaired cardiac or renal function was accepted in the treatment trial with procainamide. The serum creatinine was always less than 1.3 mg% 1.06 mg% on an verage. The mean age of the patients was 53.2 years (range 22 71) and the mean weight 74.6 kg (range 50–105).

In group of 16 patients the plasma concentrations and the biological half life of procainamide were studied after single dose of either 3 250 mg in ordinary tablets or 2 400 mg in sustained-release tablets.

In group of 7 patients the plasma concentrations with 2 400 mg t.i.d. in sustained-release tablets were studied during 3-day trial. Another group of 7 patients served as reference group taking ordinary tablets 3 250 mg t.i.d. for 3 days. Both groups consisted of 5 men and 2 women. The mean values in these groups were 50 and 57 years for age, 75 and 77 kg for weight, 1.02 and 0.96 mg% for serum creatinine. Five of the

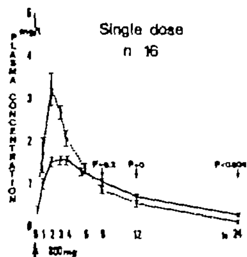


Fig. 1. Mean and S.E.M. values of plasma procainamide concentrations after a single dose in 16 patients. —— sustained-release tablets, – ordinary tablets of procainamide.

patients on the sustained-release tablets and an extra patient were observed for 4 weeks when using the same dosage.

In a group of 16 patients the plasma concentrations were similarly observed with a larger dose, 3 × 400 mg t.i.d., in a 3-day trial. In these patients, who had a relatively constant number of VPBs before the treatment (on an average 13.2, S.D. ± 7.8, range 1.3–29.8 VPBs/min) the antiarrhythmic effect of procainamide was also noted.

Tablets made. The procainamide was made into a sustained-release tablet with the aid of resin, a product of polymerization of methacrylic acid and methacrylic esters, and solidified oil. The tablet was film-coated in order to make it tasteless. The ordinary or standard tablet was Cardiorytmin® (Star, Tampere).

In vitro determination of the release rate. The dissolution fluids used were made up according to the U.S.P. XVIII with the following modifications.

1st hour artificial gastric fluid without enzymes, pH 1.2; 2nd hour artificial intestinal fluid without enzymes, pH adjusted with hydrochloric acid to 4.5; 3rd hour artificial intestinal fluid without enzymes, pH adjusted to 6.8; 4th–7th hour artificial intestinal fluid without enzymes, pH 7.4.

In the study 500 ml of dissolution fluid was used for one tablet. As dissolution apparatus the JEL apparatus (J. Engelhmann, Ludwigshafen) was used. The procainamide hydrochloride content of the fluid was determined according to the method described by Bratton and Marshall (4). After the 7th hour the procainamide content of undissolved matrix was also measured. The release rate of the tablets of each batch was also determined by drying and weighing the undissolved matrix of a sample tablet after every hour for 5 hours.

Plasma procainamide concentrations. Ten ml venous blood was centrifuged after coagulation and the separated serum was kept deepfrozen until the procainamide content was measured. In a comparative series both serum and plasma concentrations were determined and found to be identical. In this context the concepts of plasma and serum concentrations are used as synonyms. The method of Mark et al. (16) was used. This method requires extraction of procainamide into benzene and reextraction into an acid aqueous solution. After diazotization and coupling with N-(1-naphthyl) ethylenediamine the optical density was measured at a wavelength of 550 mμ in a spectrophotometer. The method is comparatively simple, highly reproducible and sensitive to 0.05 mg/l.

Counting of ventricular premature beats. An ECG recording for 1 1/2 min (2.25 m strip at 25 mm/sec) was made every 4th hour on 16 patients, who had been lying supine in their beds for at least 10 min. The number of VPBs in the strips was later counted by the same staff physician.

RESULTS

In vitro studies. The cumulative dissolution rate of the sustained-release tablets was slow and at 8 hours 12% of the original quantity was still found in undissolved matrix. The dissolved quantity in one batch was 37% at 1 h, 54% at 2 h, 63% at 3 h, 71% at 4 h, 77% at 5 h, 82% at 6 h and 86% at 7 h. In two other batches of tablets the dissolved quantity at 6 h was 77% and 78%, the dissolution rate being somewhat slower. When the dissolution rate was determined only by drying and weighing the undissolved matrix, almost identical results were obtained: 38% at 1 h, 58% at 2 h, 66% at 3 h, 74% at 4 h and 78% at 5 h.

In vivo biological half-life. In the group of



Fig. 2. Mean and S.E.M. values of plasma procainamide concentrations in a 3-day trial in two groups of seven patients and in a month's trial in six patients. The time of administration of the drug is indicated by arrows. Other symbols as in Fig. 1.

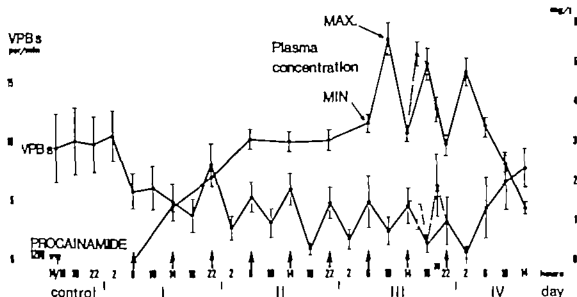


Fig. 3. Mean and S.E.M. values of plasma procainamide concentrations in 16 patients in 3-day trial with 1 200 mg t.i.d. of procainamide in sustained-release tablets (arrows indicate time of administration) and mean and S.E.M. values of VPBs in 12 of these 16 patients before, during and after the treatment period.

16 patients the mean half-life of the standard tablets was 3.1 hours (S.D. ±0.63, range 2.0–4.1). With the sustained-release tablets in the same patients the half-life was 6.7 hours on an average (S.D. ±1.5, range 4.6–9.3). The mean values for plasma procainamide levels are presented in Fig. 1 for the single doses. The peak concentration was significantly higher with the standard tablets, but there was no statistically significant difference at 8 or 12 hours, at which times the concentrations were very small.

3-day treatment periods. During the 3-day treatment with the sustained-release tablets 2 × 400 mg t.i.d., the peak mean plasma concentration was 3.7 mg/l and the minimum mean concentration before the new dose approximately 2.0 mg/l at 24 and 36 hours. The plasma level was always maintained above 3.0 mg/l for about 3 / hours after administration of the last tablet (Fig. 2). The mean peak level at 2 hours after administration of the standard tablets 3 × 250 mg t.i.d. on the 3rd day was 4.6 mg/l. The mean minimum level was only 1.14 mg/l.

Sustained release tablets in a larger dose, 3 × 400 mg t.i.d. were given to 16 patients. The results are presented in Fig. 3. The mean minimum level was now 3.2 mg/l at 24 and 36 hours. The peak conce trations were about 5.4 mg/l on an average. The plasma levels were maintained above 3.8 mg/l until 6 hours after the last tablet.

The plasma concentrations were not clearly dependent on the dose of the sustained-release tablets calculated per kg b.wt. as can be seen in Fig. 4. The highest plasma level during the 3-day trials was 98 % greater on an average than the lowest steady state level when the dose was 800 mg t.i.d. and 70 % when the dose had been 1 200 mg t.i.d. This important range was 424 % with the standard tablets in a dose of 750 mg t.i.d.

The number of VPBs decreased in the 16 patients in whom they were observed. The reduction after 24 hours treatment was on an average 24 % with the minimum concentration and 45 % with the maximum concentration. The mean reduction at maximum concentrations was statistically significant ($p < 0.01$) as compared with pretreatment or minimum concentration periods. The difference between pretreatment and minimum conce tration periods was not significant in the whole group of patients. In Fig. 3 the mean numbe of VPBs of 12 patients is presented. Three patients with mean pretreatment n mber of VPBs of 8.0, 19.8 and 29.8/min were omitted because procainamide showed no clinical

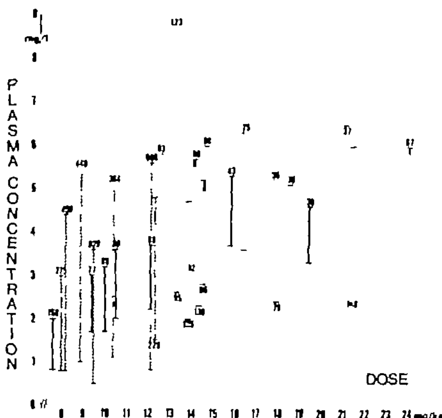


Fig 4 Minimum and maximum values of the plasma procainamide concentration of 30 patients during the day of treatment with an 8-hour dosage schedule in relation to the dose (mg/kg b.wt.). The figures show the percentage by which the maximum concentration exceeded the minimum level. *Symbols as in Fig 1*

in any concentration in these cases. The fourth patient had only 1 3 VPBs/min during the pretreatment period. The other 12 patients illustrate the variation in the number of VPBs closely and inversely following the plasma concentration after each tablet, beginning with the 3rd dose at 16 hours. The VPBs were abolished in most patients from the 2nd to the 6th hour after each tablet.

Plasma concentrations after 4 weeks. In 6 patients the plasma levels of procainamide were determined after 1 2 and 4 weeks of treatment with 800 mg t.i.d. in sustained-release tablets. There was no statistically significant difference between the first steady state level and the following three levels, which varied between 2.3 and 2.9 mg/l (mean minimum concentrations) (Fig. 2)

DISCUSSION

The results of this study confirm earlier observations (12) that the dosage interval with procainamide should be equal to the biological half life which was found to be 6.7 hours with the new sustained-release tablets and 3.2 hours with the ordinary tablets. With the ordinary tablets a dosage interval of 8 hours yielded peak plasma concentrations which were about 400% higher than the minimum steady state concentrations. Great fluctuations may cause toxic effects to appear at peak levels and the concentration may again be ineffective with reoccurring arrhythmias prior to the next dose (3). When the same quantity of procainamide was given in slowly dissolving tablets the range between peak and minimum concentrations was only about 100% and the minimum level was significantly higher than with the ordinary tablets. When a higher dose was administered, 1 200 mg t.i.d., in sustained-release tablets the minimum steady state concentration of 3.2 mg/l was attained after 24 hours and the peak concentrations were about 5.4 mg/l on an average or 70% higher than the minimum level. Koch-Weser and Klein (14)

recommended a daily dose of 50 mg/kg procainamide in regular tablets, to be given every 3rd hour With 3 0 g daily they found that plasma concentrations fluctuated between 4 and 7 mg/l with an acceptable difference of 34% In our study the daily dose of 3 6 g was on an average 49 mg/kg. It can be calculated on the basis of these findings that the optimum dosage of procainamide in the sustained-release tablets ould be 1 0 g q.i.d. for an a erage patient (0 8–1 2 g depending on the body weight).

The steady state plasma concentrations could, however not be predicted on the basis of the oral dosage or the weight of the patient. Inter individual differences were large. This is a common finding in patients with a myocardial infarction and a secondarily impaired renal function because of the decreased rate of renal excretion of procainamide. The gastrointestinal absorption of the drug is also slow and highly variable in these patients. In a study of 91 patients, most of them with acute myocardial infarction, Koch-Weser (12) found differences up to 400% between average plasma concentrations at a given daily dose. The absorption was less than 50% in 4 of 15 patients (12) The sustained-release tablet used in our study might even have too slow an absorption in some patients, resulting in greater interindividual variation The coefficients of variation in 16 patients were, however the same with ordinary (21%) and sustained-release tablets (23%) It is obvious that the dosage for any individual patient should be based on plasma level determinations, which fortunately are relatively simple to perform (5 13). No cumulative tendency was seen in the plasma levels of the 6 patients who used the sustained-release tablets for one month The plasma levels were individually constant when the clinical state of the patient was stable

The number of VPBs decreased significantly in 16 patients in whom they were cou ted from a 1 / min ECG recording every 4th hou Plasma procainamide determinations were made simultaneously On the 3rd d y both determinations were made at 2-hour intervals and the number of VPBs was found to be smallest 2 hours after the peak level of concentration. W th increasing plasma levels the number of VPBs fell significantly and fluctuated as mirror image of the plasma level. In terms of a complete lack of VPBs the rate of success was high, but no plasma level was effective in all cases. Koch-Weser and Klein (13) reported their experience in 142 patients treated with the regular short acting tablets of procainamide. They found that about 90% of the patients were without ar rhythmias at a plasma level of 8 mg/l. Their study included only patients in whom procainamide was effective (even if with a high dosage) Plasma levels of less than 4 mg/l were effective in less than half of the patients.

Procainamide has a relatively narrow therapeutic range. Symptoms and signs of overdose appear if a plasma level of 16 mg/l is exceeded (12, 13). Toxic concentrations may cause an extreme conduction delay in the atrium, ventricle and especially in the A V node, and they may enhance automaticity and multifocal pacemaker activity Myocardial contractility may be depressed, resulting in congestive heart failure or hypotension (2). Other side-effects in connection with dose are anorexia, nausea and vomiting, occasional mental depression psychosis and convulsions (3 10) In our study a woman (excluded from the series) who weighed 50 kg and whose serum creatinine was 1.5 mg% attained on the 3rd day with a smaller dose, 800 mg t i d a peak concentration of 16 6 mg/l, the minimum concentration being 13.2 mg/l. The patient had nausea and psychotic symptoms. Another woman with a serum level of only about 6 mg/l had nausea, visual hallucinations and aggressive be haviour These two patients had no other medicament. With the exception of one case of ur ticaria and drug fever no other major acute undesirable effects were found in the 47 patients.

The association of an illness resembling systemic lupus erythematosus (SLE) with procainamide treatment has been well established since Ladd's description in 1962 (15). Fakhro et al (7) reported this syndrome in 15 of less than 50 patients who had used procainamide for prolonged periods Hope and Bates (9) found this syndrome in 3 of 61 patients who had been taking procainamide for longer than one month. The incidence of the disease increases with the duration of exposure to procainamide. It is greater with large doses, even if it has been reported with as low a daily dose as 0 5 g in some patients (9 18) With large doses of long acting tablets the incidence may become still

greater Some 50–80% of patients who have been receiving procainamide for a long time have circulating antinuclear antibodies (9 20) The LE cells are diagnostic for the disease. ESR has been found to be inconsistently elevated (18) and is not reliable as a screening method. The syndrome induced by procainamide is usually less severe than the spontaneous SLE disease and is generally without renal involvement (6). Arthritis, pleurisy and pericarditis are common. They are usually reversible when the administration of the drug is stopped, but they may occasionally persist for months (1 6). For very long-term usage it may be advisable to replace procainamide by sustained-release tablets of quinidine. The electrophysiological effects of both drugs are for practical purposes indistinguishable (21) and the syndrome similar to SLE has only once been reported in connection with quinidine therapy (11).

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UNUSUAL PATTERN OF HEPATIC ALKALINE PHOSPHATASE ACTIVITY AND RENAL CARCINOMA

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Abstract. Two cases of renal carcinoma associated with hepatic dysfunction in the absence of hepatic metastasis are presented. A moderately elevated activity of serum alkaline phosphatase was found. Liver biopsy specimens disclosed normal morphology but an abnormal localization of alkaline phosphatase activity was found adjacent to the canaliculi in the entire hepatic lobules. Serum alkaline phosphatase and platelet levels became normal after extirpation of the tumour. It is concluded that the canalicular phosphatase activity is in some way induced by humoral tumour agent. It is of clinical importance to realize signs of hepatic dysfunction as paraneoplastic manifestation and, in known tumour cases, not to interpret them as unequivocal symptoms of liver metastases.

Renal adenocarcinoma is a puzzling tumour occasionally presenting with paraneoplastic symptoms from many organ systems. During the last decade several reports have appeared about cases associated with hepatic dysfunction in the absence of hepatic metastasis (4). Elevated serum alkaline phosphatase levels have been reported in these subjects. In this report we present two cases of renal carcinoma with a histochemically unusual localization of increased alkaline phosphatase activity and an elevated serum alkaline phosphatase level.

CASE REPORTS

Case 1

A 34-year-old woman had repeated bouts of tonsillitis and cystitis. She complained of tiredness, headache, pains in her muscles and joints, and sometimes she was subfebrile. A persistently elevated ESR was found. In spite of comprehensive investigations the diagnosis was obscure. Steroid therapy was administered without certain effect on the ESR. The patient was then admitted to the Medical Clinic of Malmö General Hospital.

Physical examination disclosed persistent tachycardia BP 130/80 mmHg and no palpable liver or spleen. Pathological laboratory findings included an ESR of 140 mm/h, hypochromic anaemia of 8 g/100 ml, elevated platelet counts up to 530 000. A sternal marrow aspirate showed relative increase in megacaryocytes. Serum protein electrophoresis gave the picture of an inflammatory reaction. Fibrinogen was highly increased (above 1 g/100 ml). Prothrombin-proconvertin activity was decreased to 50%. Serum alkaline phosphatase activity was increased (17 Bach units, normal range 2–8). Serum γ-glutamyl transpeptidase was at the upper normal limit, serum transaminases and bilirubin were normal. A percutaneous liver biopsy specimen showed normal morphology in conventional stainings, but among series of enzyme histochemical stainings alkaline phosphatase revealed widespread canalicular activity which is abnormal for healthy human liver (Figs. 1 and 2). (For methods see refs. 1, 2.) Liver scan disclosed no size increase and no abnormal activity. A lymphoglandular specimen obtained by mediastinoscopy, muscle specimen and biopsy specimen from the right temporal artery showed no pathological changes. Comprehensive X-ray examinations were negative.

An abdominal and renal angiography was then performed, and tumour was revealed in the lower part of the right kidney. Nephrectomy was done. The tumour was 5.5 cm in diameter. The microscopic appearance was that of renal adenocarcinoma with predominance of clear cells. On the 4th postoperative day serum alkaline phosphatase activity was still increased (19 Bach units). Four months later ESR was 11, platelet count 202 000, prothrombin–proconvertin normal and serum alkaline phosphatase 5. The patient is by now well and free from her pains in muscles and joints. Besides oral iron therapy and 5 mg diazepam (Valium Roche) for the night the patient got no medicine when the liver biopsy was performed.

Case 2

A 54-year-old man was operated upon in 1965 because of left-sided renal adenocarcinoma. The tumour was 5 cm in diameter and histologically composed of mixed clear and granular cells. The tumour was disclosed by urography and renal angiography was performed because of persistent cystopyelitis. No hypertension was present, liver and spleen were not palpable. ESR was 30 mm/h, and there was no anaemia. Platelet count or liver tests were not performed. Postoperatively the patient was treated with cobalt irradiation.

less than 20 of the 600 liver biopsy specimens. Most of the patients with this finding have been found to have malignant and/or "collagen" diseases. A study of these subjects is being prepared by one of us (I. H.).

The increase in canalicular alkaline phosphatase activity might well be due to a remote influence upon the hepatocytes by the renal tumour and possibly to some agent secreted by the cancer cells (4). A similar influence has been shown biochemically on lysosomal hydrolases of hepatic tissue, their activity being increased in at least some subjects with renal carcinoma without liver metastases (5). Stainings for acid phosphatase and β-glucuronidase activity were performed in the liver specimens of the present two cases. One case showed strong acid phosphatase activity of hepatocytic lysosomes while the β-glucuronidase activity was weak in the other case the findings were reversed. Agent(s) produced by the tumour cells might be supposed to enter hepatocytes through endocytosis and be at least partly digested by acid hydrolases, and metabolites might be secreted into the bile demanding canalicular alkaline phosphatase activity.

Renal carcinoma belongs to a group of tumours in which tumour cells often have alkaline phosphatase activity (8) as in our case 2. The so-called Regan isoenzyme, a placental type alkaline phosphatase, has been reported in the serum from a subject with renal carcinoma (4). The molecular type, i.e. placental, liver, intestinal or osseous, of the tumour and serum alkaline phosphatase in the two patients is at present unknown. Therefore it cannot be concluded with any certainty whether the serum enzyme had its origin in the hepatic canaliculi, the hepatic sinusoids, or in the tumour. Presumably the normal serum alkaline phosphatases, as well as those causing elevated serum activity in liver diseases, have their origin in the hepatic sinusoids.

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THE DIAGNOSTIC SIGNIFICANCE OF A HIGH ASAT/ALAT (GOT/GPT) RATIO IN PATIENTS WITH VERY HIGH SERUM AMINOTRANSFERASE LEVELS

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Abstract. A consecutive study of 160 patients with very high (>500 U/l) serum ASAT activity has revealed that this was most commonly due to acute hepatitis, circulatory disturbances, or malignant liver disease. In acute hepatitis the ASAT increase was usually accompanied by a similar rise in ALAT. An ASAT/ALAT ratio >3 was almost entirely restricted to patients with circulatory disturbances or malignant liver disease. The latter diagnosis should thus be suspected in patients with markedly increased serum aminotransferase activity and an ASAT/ALAT ratio >3 without evidence of circulatory disturbances.

Much has been written about serum aminotransferases in liver diseases. It is generally accepted (1) that very high aminotransferase levels in serum are seen most commonly in acute hepatocellular damage, e.g. viral or toxic hepatitis. It is also commonly stated that in these cases the increase of alanine aminotransferase (EC 2.6.1.2, ALAT GPT) is greater than that of aspartate aminotransferase (EC 2.6.1.1 ASAT GOT). In diseases of the biliary tract and in chronic hepatocellular disease the aminotransferase rise is generally reported to be less pronounced. Varying experiences are reported on the ASAT/ALAT ratio in diseases of the biliary tract, whereas in liver cirrhosis the ASAT/ALAT ratio is usually reported to be >1.

Clinical experience of some surprising causes of very high serum aminotransferase levels initiated the present consecutive study of patients with very high (>500 U/l) serum aminotransferase levels. Our intention was to clarify the underlying disease and the ASAT/ALAT ratio in the different disease groups.

MATERIAL

During a 16-month period in 1970–72, 160 patients at Sahlgren's Hospital, Gothenburg, were observed to have ASAT values >500 U/l (normal value <17 U/l). In 122 patients it was possible to establish with reasonable accuracy the underlying cause of the increased aminotransferase levels.

Since our investigation indicated characteristic aminotransferase patterns in hepatic malignancies, it was considered of interest to make a further study of such patients. We therefore investigated the aminotransferase patterns in all patients with primary liver cancer observed at the hospital during 1959–71. Serum ASAT levels higher than normal were recorded in 83 of the 143 patients observed during this period.

It was also considered of interest to compare the aminotransferase levels in these cancer patients with those of patients with other chronic liver diseases. For this purpose we studied the aminotransferase values of all patients with an autopsy diagnosis of liver cirrhosis observed at the hospital during 1961–68. Serum ASAT levels higher than normal were recorded in 13 of the 224 cirrhosis patients.

The ASAT/ALAT ratio was calculated on the values from the day with the highest recorded ASAT value. ASAT and ALAT were determined by conventional methods based on continuous registration of the oxidation of NADH at a reaction temperature of 30°C. The upper reference value is 17 U/l for both enzymes.

RESULTS

Patients with acute hepatitis represented the largest disease group (49 patients) with serum ASAT levels >500 U/l (Fig. 1). Circulatory disturbances (prolonged hypotension, shock, pronounced cardiac failure) without evidence of myocardial infarction were another common cause of very high serum ASAT levels (29 patients) as was myocardial infarction with circulatory failure (14 patients). Hepatic tumours also accounted for a great part of the recorded cases (13 patients).

The pronounced increase of the serum ASAT levels was accompanied by a similar increase of

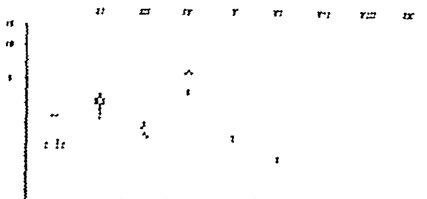


Fig. 1. ASAT/ALAT ratio in patients with serum ASAT > 500 U/l. I, Acute hepatitis. II, Circulatory failure without myocardial infarction. III, Circulatory failure with myocardial infarction. IV, Malignant liver disease. V, Biliary tract disease. VI, Toxic liver damage. VII, Chronic active hepatitis. VIII, Liver trauma. IX, Skeletal muscle necrosis.

the serum ALAT level in acute hepatitis (Fig. 1). An ASAT/ALAT ratio >3 was thus not observed in any of the patients, the highest ratio recorded being 2.3, whereas a ratio <1 was observed in no less than 24/49 hepatitis patients. An ASAT/ALAT ratio >3 was almost entirely restricted to patients with circulatory failure or malignant liver disease, the latter diagnosis being a surprisingly frequent cause in view of the relative rareness of the disease. The hepatic malignancies were both primary and secondary and of different origin without dominance for any specific group. In three patients circulatory disturbances presumably contributed to the elevated aminotransferase level.

Since these data indicated that a high ASAT/ALAT ratio in patients with pronounced serum ASAT elevations could be an indicator of hepatic malignancy, the study was extended to comprise a series of patients with primary liver cancer which was compared with a series of patients with liver cirrhosis without autopsy evidence of hepatic malignancy. Five patients with primary liver cancer and 14 with liver cirrhosis had serum ASAT values >500 U/l. The very high ASAT/ALAT ratios observed in the hepatic malignancy group in the consecutive study were found also in the primary liver cancer group (Table I). The ratio ranged between 3.1 and 9.0 with a mean of 5.54, which was significantly higher than the mean of 2.05 observed in the liver cirrhosis group. However, 2 of the 14 cirrhosis patients with ASAT >500 U/l had ASAT/ALAT ratios higher than the lowest ratio observed in the liver cancer group with similar levels of ASAT. The discriminatory ability of the ASAT/ALAT ratio was thus not complete.

The mean ASAT/ALAT ratio in the patients with ASAT <500 U/l was also significantly higher in the liver cancer group, but here the difference was very small.

The observed differences in ASAT/ALAT ratio between cancer and cirrhosis patients were not related to differences in ASAT levels, as is evident from Table I.

Table I. *ASAT/ALAT ratio and serum ASAT value (mean ± S.E.M.) in patients with liver cancer or liver cirrhosis without liver cancer*

	Primary liver cancer	Liver cirrhosis without liver cancer	*p*
ASAT >500 U/l	5.54±1.39 n=5 (ASAT 748 ±41)	2.05±0.34 14 (ASAT 1 18 ±41)	0.05
ASAT 50–500 U/l	2.60±0.19 n=78 (ASAT 134±8)	2.16±0.11 n=116 (ASAT 134±9)	<0.05

DISCUSSION

The most interesting conclusion that can be drawn from this study seems to be that a high ASAT/ALAT ratio (>3) in a patient with a serum ASAT >500 U/l is highly suggestive of hepatic malignancy. As a rule it should be possible to exclude the most common differential diagnosis, circulatory disturbances with or without myocardial infarction. As could be expected, the observed high ASAT/ALAT ratio was a sign of advanced disease: only two of the nine patients survived for more than one month.

The reason for the predominant increase of serum ASAT in patients with liver tumours is not

clear Pietschmann et al. (2) have observed a decreased activity of ASAT in primary as well as secondary liver cancer tissue. Since ASAT is located primarily in the mitochondria, they suggest that their observation is explained by the known fact that cancer tissue contains considerably less mitochondria than normal tissue. Our own observations suggest that an increased leakage of ASAT may be an additional explanation of the low ASAT activity in hepatic tumours. However the cause of the predominant leakage of ASAT is completely obscure to us.

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A CLINICAL SYNDROME WITH INBORN DEFECT IN ERYTHROPOIESIS DYSPLASTIC KIDNEYS EYE LESIONS MALFORMATION OF THE TEETH AND IMPAIRED HEARING

A New Syndrome in a 28-year-old Woman

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Abstract. A 28-year-old woman is described, having defective erythropoiesis, kidney dysplasia, minor skeletal defects, progressive loss of vision, abnormal development of the teeth and slight impairment of hearing. Her disease is most probably inherited. It started in the first years of life with thirst and weakness. From the age of 20 she had to be given blood transfusions. Kidney function has deteriorated very slowly. For many years the only feature of impaired renal function was low specific gravity of the urine. Serum creatinine is now 2.6 mg/100 ml. Loss of vision of the left eye was noticed very early and this eye was removed when she was 12 years old because of secondary glaucoma. The other eye was later affected, and she is now blind. Very recently slight neurogenic loss of hearing has occurred on the left side. Dental development was abnormal. The skeletal defects of genu valgum and pes excavatus were moderate. The mental, endocrine and sexual development have been normal. Her father and mother are healthy but they have reduced PAH and inulin clearance, indicating slight impairment of renal function. Renal biopsy from the patient revealed kidney dysplasia with smooth muscle in the parenchyma and lack of Henle loop. The cause of this syndrome and the mechanism by which all these different organ disorders can be linked together is completely unknown. To our knowledge this syndrome has not been reported previously.

Rare clinical syndromes receive great attention because they may throw light on biochemical and pathophysiological problems. Patients with symptoms and signs similar to those of such syndromes, but not definitely typical of one of them, are usually not classified as belonging to that particular syndrome and are therefore not published. In view of the enormous range of biochemical polymorphisms, however, it would not appear surprising that a genetic defect responsible for a certain syndrome may have different expressions in humans.

A 28-year-old woman with inborn defects in several organs, slowly increasing in severity, is described. The cause of these defects has not been found, but we nevertheless feel that this case represents either a new clinical syndrome or a peculiar variant of a known syndrome. Publication might help to obtain knowledge about similar cases.

CASE REPORT

A girl, 4 months of age, was admitted to the Pediatric Department, Rikshospitalet, because of an anemia with Hb 11.7 g/100 ml and erythrocytes 5 mill./µl. Repeated trials with oral iron were ineffective. At the age of 2 she was given thyroid preparation because of weakness, constipation and some retardation in ability to walk. Post or propter she became more vital, but some weakness persisted. At that time her mother noticed that she often was remarkably thirsty.

At the age of 8 the thyroid treatment was stopped, as there was no evidence of endocrine dysfunction. At this time the examination disclosed slight anemia (Hb 9.7 g/100 ml, erythrocytes 4.6 mill./µl and serum iron 109 µg/100 ml). On ophthalmological examination amblyopia was discovered in the left eye. The visual acuity was probably slightly reduced on the right side but the eye ground was described as normal. The urine was normal. The dentist reported atypical development of her teeth.

She was readmitted when 12 years old. Just prior to that her left eye was removed due to painful glaucoma. The girl felt [illegible] tired, complained of thirst and had episodes of [illegible] in the abdomen. The anemia was unchanged and the specific gravity of urine was 1.008. Her [illegible] and it was noticed that she had moderate genu valgum and pes excavatus. Caries of

periodontia were a serious problem, and a peculiar resorption of some permanent teeth had started. A prominent processus maxillaris was noted. EEG showed possible changes in deep medial structures. Air-encephalograph disclosed some enlargement of lateral ventricles and 3rd ventricle and it concluded that she might ha lesions in the egion of the hypothalamus. However her IQ and sexual development later on era normal Ophthalmological control disclosed itreous opacities and some minor changes in the macular region. I pyelogram was normal Ther was no evidence of urinary tract infection

The patient as admitted to Medical Department A at the ge of 18 She had prior thereto been treated for pain in the ey and the fundus showed exudations and bleeding. An attack of abdominal pain as misinterpreted as appendicitis, and a normal appendi as emoved ithout complications As found earlier the Hb content in the erythrocytes as educed despite normal serum iron Hb 8.8 g 100 ml and erythrocytes 4.5 mill / μl. Serum creatinine 1 6 mg/100 ml and specific gra ity of urine 1 009

The following course was a slowly progressing chronic disease She became blind and had no benefit of extraction of cataract Her thirst became less (unfortunately!) and f om the ge of 19–20 she had to be given blood transfusions. Due to her loss of vision and tendency to dizziness she does not tolerate Hb alues below 8 g/ 100 ml. In the last 3 years she has had ttack of right sided abdominal colic starting in the right lumbar egion and irradiating to the right inguinal area. The abdomen was tender in the egion of the right kidney and the clinical condition as suggesti of urolithiasis. Urological examination, examination of urine and retrograde pyelograms er normal. On one occasion she also had similar in in the left side Spasms in the bundles of smooth 'e in the kidneys might perhaps be the cause (see). E idence of epilepsia has not been found. EEG 3 only slight generalized cerebral dysrhythmia.

The patient has now finished her training at the School for the Blind During this training her normal intellectual f nctions er evident.

CHARACTERIZATION OF THE VARIOUS ORGAN INVOLVEMENT

Anemia. The first complete hematological investigation was performed at the age of 18 The anemia has been hypochromic, ith MCH of 23–27 γγ her peripheral blood showing slight anisocytosis and few Target cells, otherwise normal red cell morphology In the last year the MCH has approached normal alues. Her bone marrow has been cellular showing an increase of normoblastic erythropoiesis and no signs of ringed sideroblasts. Due to the transfusions there has been an increased amount of hemosiderin in the marrow Thrombopoiesis and granulopoiesis are normal, and there has alway been normal WBC and differential counts, normal platelet counts and normal score for leucocyte alkaline phosphatase Both megacaryocytes and platelets appear normal, and neither clinically nor by laboratory test has any bleeding tendency been found. Despite the hypochromic anemia her serum iron has been normal or slightly elevated (180 μg 100 ml) with transferrin in the normal range (280 μg 100 ml) The reticulocyte counts wer usually 3–20‰. Serum haptoglobin, LDH and bilirubin alues er normal on several occasions, and the half-lif of Cr^{51}-tagged own erythrocytes as 28 and 3, days, examined with 7 years leters t. The erythrocytes ha normal osmotic fragility The spleen has never been enlarged. Serum B_{12} as normal, but folic acid low (serum 1.5 ng/ml and RBC 77 ng/ml). Hb was type A on electrophoresis. Ther was no e idence of deficiency in intra-erythrocytic enzymes. (Elaborate studies performed by Dr M Hjelm, Uppsala, Sweden) Agarose gel electrophoresis of serum showed normal transferrin band. Coproporphyrins in erythrocytes wer 0.1– 0 and protoporphyrins 0.4–6.0 μg/100 ml (normal alues). The excretion in urine of these substances was normal. Serum GOT and GPT and alkaline phosphatase lues er ithin normal range, but in the last 3 years there has been a tendency to slightly ele ted alkaline phosphatase levels. W ha not had the possibility to measure hemsynthetase Sucrose test and examination for hemosiderin in urine er normal Serum electrophoresis, immune quantitation of immunoglobulins in serum cholesterol, triglycerides, phospholipids, and phospholipid fractions in plasma and erythrocytes revealed no abnormality Electrolytes in serum er als) normal, but in the last year an increase in phosphorus to 5 1 mg 100 ml has occurred.

Kidneys. Lack of ability to concentrate the urine was the first sign of kidney disease. For many years the urine was otherwise normal. Episodes of urinary tract infections in the last years might be responsible for the slight proteinuria now present. She has als) had normal BP At the ge of .5 and 28 the following enal function studies ere performed in April 1968 inulin learance as 33 ml/min, renal plasma flow 328 ml min, enal blood flow 444 ml min, oxygen consumption 6.4 ml/min and citrate clearance 9.27 ml/min (normal values for these parameters are 120–140 550–700 900–1 200 17 22 and 30-35 respectively). In April 1971 3 years later the studies evealed a progressi impairment of enal function. Inulin clearanc 15 renal plasma flow 203 enal blood flow 261 xygen consumption .4 and citrate clearance 4 66. In April 1968 the extraction rate of PAH was 54% (normal 88–94%). (The results are erage values of 2 3 periods of 15–20 min duration) The pyruvate-lactate ratio as normal. These studies indicated that the glomerular invol ement as mor advanced than the damage to the renal ascular ystem. Early polyuria and better conservation of the glomerular ultrafiltra n and vascular system than of the tubular function, late period by extraction rate of PAH t the first examination indicated a mor ad anced tubular damage These results cor esponded well ith the histopathological findings in the enal biopsy I pyelograms has demonstrated kidneys of approximately normal size, ith normal pe is and drainage. Light microscopic examination of the kidney biopsy revealed bundles of smooth muscles in th renal parenchyma, and the loop of Henle could not be found (Fig. I). Amyloid deposits were bsent Mos of th tubuli seen in the preparation had the ppearanc of collecting tubules. The embryological evaluation of kidney





Fig. 1. Hematoxylin-eosin stained sections of kidney biopsy

biopsies was performed by Dr H. Stalsberg, Department of Pathological Anatomy University Hospital, Ullevål Hospital, Oslo.

Ocular involvement. A divergent squint of the left eye was present from 1 year of age. When she was 7 years old, unilateral left-sided myopia of −13D was found, while the right eye was emmetropic. Visual acuity at that time was: right eye: 6/6 left eye: counting fingers. At the age of 11 posterior subcapsular opacities and vacuoles in addition to vitreous opacities were found. At that time she had iridocyclitis with "iris bombé" and secondary glaucoma. The left eye was removed due to pain, but the right was normal. However, a few months later minor vitreous opacities were found, the optic disc was edematous nasally and the macular region was slightly affected. At the age of 18 her right eye was examined because of floating opacities. There was a slight uveitis, posterior capsular opacities of the lens, retinal hemorrhages and soft and hard exudates. There was a secondary glaucoma caused by a low grade chronic uveitis. Gradually in the course of years, a posterior cataract developed and the lens was removed. She is now practically blind. Histologically the left eye showed features of chronic uveitis. The iris was atrophic, with infiltration of lymphocytes and some mast cells. A marked gliosis was found in retina, there were degenerative changes of the ganglial cells, hemorrhages and exudation of lymphocytes and granulocytes. The optic nerve was normal. The histological examination of the eye was performed by Dr K. Arnesen, Department of Pathological Anatomy University Hospital, Ullevål Hospital, Oslo.

Skeleton. At the age of 8 normal structure and growth zones were found on X-ray of cranium and feet. Later she grew normally but has a moderate degree of genu valgum and pes excavatus. Her palatum durum is high and narrow. X-ray of cranium is normal.

Teeth. Already as a child it was noted that the processus condylaris was very prominent and that her teeth were abnormal. She got her first tooth at 9 weeks and had all her teeth at 9 months. The permanent teeth appeared already at the age of 3 and were complete at the age of 6. At the age of 12 it was noted that an abnormal resorption of the roots had started. Marked periodontitis and caries were present and she had lost several permanent teeth. The malformation involved the mesodermal part of the tooth and it was described as to some extent similar to that in osteogenesis imperfecta. One of the most prominent signs was the gracile, curved

roots. The teeth had normal density and colour. Resorption of the alveolar process was marked.

Hearing. When a few years old she had some episodes of otitis media. In the last 3 years she has noticed slight difficulty in hearing. At the age of 26 audiological examination was performed. Clinically the ear-nose-throat examination was normal. On the left side a neurogenic loss of hearing was found, cochlear type audiogram, similar to that found in patients with Mb. Menière.

Other examinations. At 26 years of age she had a period of arthralgia in upper extremities and elevated ESR. This subsided without treatment. Examinations of chest organs, liver and gastrointestinal tract have been normal. She has a tendency to gain weight. Except for haemosiderin accumulation, the liver biopsy is normal. Her sexual development has been normal, and her menstruation was regular except for minor irregularities at the age of 25. Chromosome studies were performed at two different laboratories, both with normal count and appearance. She has several times been examined for possible endocrine dysfunction, but always with normal result. Uptak of isotope-I was normal, and excretion of corticosteroids and aldosterone in urine has been normal. Both peroral and I load with glucose ere normal. Wound healing is normal with normal skin. Acid-base disturbances have never been recorded. A recent EEG revealed slight generalized cerebral dysrhythmia, otherwise normal. Chromatography of urine did not reveal abnormal metabolites, and elevated levels of known metabolites have not been found on multicomponent analysis.

Family studies. Both father and mother were clinically healthy and had normal hematological status, electrolytes, urine, and normal serum creatinine. However, both inulin and PAH clearance wer abnormal in both parents. The mother was examined in 1968 and had inulin clearance 71 ml/min and PAH clearance of 394–363 ml/min. The father was studied in 1971 and the values were 53 and 293, respectively. His citrate clearance was 27.71 ml/min. Ophthalmological examination and hearing were normal in both parents.

Therapeutic trials. For 2 years she tried prednisone, highest dose 10 mg daily, without effect, and the local treatment of the eye lesions with steroids was also unsuccessful. Injections with various kinds of vitamins B or transfusions with fresh plasma had no effect on reticulocyte count or Hb. Neither had folic acid in 0.5 mg oral dose any effect. The silent and recurring infection of the urinary tract during the last years has been treated according to bacterial sensitivity. Despite the great number of transfusions, no transfusion reactions have occurred.

DISCUSSION

To our knowledge similar cases have not been reported previously. This syndrome may theoretically be explained as either a new inborn error of metabolism or a variant of a known inborn disease. Alport's syndrome, familial hereditary nephropathy, is characterized by early albuminuria, deafness and eye affections (1, 7, 8, 10). The histological picture of the kidney in our patient, together with the hypochromic anemia and the late albuminuria, does not fit this diagnosis. For the same reasons we could not make the diagnosis of a Fanconi anemia. The eye lesions per se might fit the diagnosis of tuberous sclerosis. However, there was no other manifestation of that disorder. Lowe's syndrome results in debility, typical EEG pattern and aminoaciduria with acidosis. These patients are nearly all boys, and a recessive defect located on the X chromosome has been proposed (4, 5). It must be questioned whether our patient, being female, might suffer from a partial expression of such a genetic defect. The histological feature of the kidney in Lowe's syndrome, however, is different from the dysplasia described in our patient.

Our patient may have an inborn error in the development of the kidneys, with the other defects as secondary manifestations. Renal insufficiency usually leads to normochromic anemia being severe only with marked depression of kidney function, and our patient had a rather slow deterioration of kidney function. Furthermore, her eye lesions could be correlated neither to kidney function nor to hypertension. Her BP remains normal. Erythropoietin levels in our patient corresponded to the degree of renal function impairment (performed by Dr S. Halvorsen, Pediatric Research Institute, Childrens Hospital, University Hospital, Rikshospitalet, Oslo). It is unlikely that all the other manifestations are secondary to a primary dysplasia of kidneys.

Our patient always had a reduced corpuscular Hb concentration and later also a reduced number of red cells. Perhaps the incorporation of iron in heme is impaired. This might indicate an enzyme deficiency. Known deficiencies in erythrocyte enzymes usually demonstrate hemolytic anemia, but our patient had normal red cell survival and no definite changes in red cell morphology. Other possibilities include abnormalities in transferrin or in binding of iron to it (2). On electrophoresis, transferrin in our patient appeared normal, and due to the many transfusions it was impossible to study the function of transferrin in this patient. Congenital dyserythropoietic anemia (3, 6) was excluded because the characteristic cytopathology of nucleated red cells was not found. Normal plasma might stimulate globin

synthesis (9), but plasma transfusions in our patient were ineffective.

Not being able to pinpoint the cause and pathophysiology of this new syndrome, we feel that this inborn error (or better system of errors) may have a common etiological background, a genetic defect. Since an asymptomatic defect in kidney function was present in both parents, it is possible that our patient is homozygous for this genetic defect.

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QUINIDINE INTOXICATION TREATED WITH HEMODIALYSIS

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Abstract. A case is described in which the ingestion of 20 g quinidine produced lethargy, respiratory distress, hypotension, anuria, loss of P waves in ECG and broadening of QRS complex. The highest serum quinidine concentration was 13.5 mg/l. Vasopressor drugs were not effective in restoring BP and hemodialysis with low concentration of potassium in dialysis fluid was done with impressive effect on the clinical state.

The main effects of quinidine intoxication are depression of myocardial excitability, weakening of myocardial contractility, prolongation of conduction time, ventricular fibrillation and peripheral vasodilatation (1, 3, 4, 7, 9, 11). Reports on treatment of quinidine intoxication recommend catecholamines or angiotensin for hypotension and cardiac pacing for prevention of asystole (4, 7, 9). In quinine sulphate intoxication, exchange transfusion (6) and peritoneal dialysis (10) have been used with success. We here report on the beneficial effect of hemodialysis in a patient with heavy quinidine intoxication induced as an attempt at suicide.

CASE REPORT

The patient was a 42-year-old housewife who formerly had attempted suicide on two occasions by "sniffing" organic solvents. It was thought that her depressive tendencies were partly caused by hirsutism for which she had been treated in the last 12 months with low doses of corticosteroids (mostly 5–10 mg prednisone a day). On Oct. 15th 1971 she ingested approximately 100 0.2 g tablets of quinidine sulphate made long-acting by coating with cellulose acetate phthalate (Kinidin Duriles®) (8), along with other tablets containing approximately 200 mg diazepam, 150 mg meprobamate and 2 g diphenhydramine.

She was admitted to our hospital 16 hours after ingestion in a state of lethargy. The skin was dry and warm and respiration normal. Rectal temperature was 36.4°C. The pulse rate was 88 and regular. BP was 150/90 mmHg.

I.v. infusion of 5% glucose was started, and the urine output was satisfactory. ECG recordings at different times are shown in Fig. 1 where corresponding concentrations of serum quinidine, measured at the Central Laboratory of Aker Hospital according to the method of Jakobsen and Cramer, and comments on clinical events are given. There was a progressive broadening of QRS complexes and prolongation of PQ and QT intervals until hemodialysis was given. In the course of the first few hours in hospital she seemed to recover and became less drowsy. But later the BP began to fall, and at 41 hours after ingestion her condition deteriorated with BP fall to 60/40 mmHg, unconsciousness and insufficiency of respiration. Repeated i.v. injections of metaraminol in 2 mg doses had no measurable effect on BP, and oliguria and later anuria supervened.

At 44.45 hours after ingestion her condition was critical. The ECG tracing as shown on the oscilloscope, was dramatically altered with lowering of the R wave, complete absence of the P wave and further widening of the QRS complex. These tracings were not recorded on paper. As the patient now was anuric, the elimination of quinidine from the organism had stopped and we had to resort to dialysis. Fearing that peritoneal dialysis would cause lung complications we decided to use hemodialysis. After cannulation of vessels on the forearm, hemodialysis was given for 6 ½ hours with 2-layered Kiil kidney of approximately 1 m² membrane area (Cuprophan 150) with a pumped blood flow of approximately 200 ml/min. Dialysis fluid, containing 1.5 mEq/l potassium and other electrolytes in usual concentrations, flowed at 500 ml/min.

The effect of hemodialysis on the clinical state was impressive. BP rose to 115/65 mmHg at the end of dialysis. Urine production again started and rose to normal levels after the end of dialysis and she regained consciousness. In the course of the next two days she recovered completely.

DISCUSSION

The clinical course may have been partly influenced by the addition of small doses of diazepam, meprobamate and diphenhydramine. The hypotension might partly have been caused by

Hours after ingestion	Comment	Blood pressure mm Hg	Serum quinidine level mg/l	ECG (Ordinat [illegible] squares [illegible] mV) (Abscissa 5 squares [illegible] sec)
00h	Ingestion of 20g quinidine sulphat			
15h	Hospitaliz tion	125/90		
17h	Good condition Lethargy Normal diuresis			
28h			13.5	
32h	Un onscious	80/50	9.5	
37h	R pirat ry distre s	75/45		
41h	Oliguria	60/40		
	Anuria Start of hemodia-lysi	60/40	12.0	ECG tracing on oscilloscope showed low voltage, grossly widened QRS comple and lacking wave
53h	End f hemodia-lysis Diur sis	115/85	9.0	
71h	Full ecovery	130/80		

Fig. 1 Sequence of the main clinical and ECG events.

insufficiency of the suprarenal glands secondary to corticosteroid treatment. The acute worsening of the clinical situation corresponded, however to marked broadening of the QRS complexes, indicating that quinidine was the relevant drug.

At the start of hemodialysis the serum concentration of quinidine was 12 mg/l on the arterial side of the artificial kidney while the level on the venous side was 11 mg/l. Calculations from these figures and the actual blood flow through the artificial kidney give an extraction of 78 mg quinidine during $6^{1}/_{2}$ hours hemodialysis. This value is so low that it cannot account for the obvious beneficial effect of the treatment. The reduction in serum quinidine concentration of 3 mg/l in the course of dialysis also corresponds poorly to the great clinical improvement.

The highest level of quinidine in the serum in our case was 13.6 mg/l. At that time the clinical state was relatively good while the dramatic worsening of the situation came when the serum concentration was 12 mg/l. In dogs it has been shown that the myocardial concentration of quinidine varies approximately 2–40 times that of the serum concentrations (9). The serum level is therefore a poor indicator of tissue concentrations and toxic effects of quinidine. As the hemodialysis treatment had very little effect on serum values, it is probable that the effect on tissue concentration has been negligible.

The best explanation of the beneficial effects of hemodialysis treatment in this case will probably be the shifts in potassium concentrations due to low potassium content in the dialysis fluid. Quinidine, by its activity on the cell membrane, is thought to increase intracellular potassium content at the expense of extracellular potassium (11). It has been shown that hypokalemia protects against quinidine intoxication in dogs (2). It is also known that muscular contractility in vitro increases as potassium concentration in the fluid bathing the muscle tissue decreases (2). During the hemodialysis with 1.5 mEq/l potassium in the dialysis fluid the patient will have a hypokalemia, and it is probably this hypokalemia which has protected against quinidine intoxication in the present case. When hemodialysis is stopped, a redistribution of potassium between intra- and extracellular compartments will usually take place in the course of a few hours. In our case there was no exacerbation of symptoms corresponding to this redistribution, but this may be due to the fact that renal function was regained.

We think that hemodialysis in this case of quinidine intoxication was life-saving. If this type of treatment is applied, it is probably important that dialysis fluid with low potassium content is used.

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Congress Announcements

The first International meeting of the society Environment and Health will be held on the Island of Cos, Greece June 23–26, 1974

Secretariat: National Research Foundation, 48 Vas. Constantinou Ave., Athens 501/1 Greece.

The VII World Congress of Cardiology will be held in Buenos Aires, Argentina, Sept. 1–7 1974.

Scientific meetings will consist of principal sessions, round tables, conferences, free communications, luncheon panels and talks with experts.

Secretary General. Dr B Malamud, Avda. Roque S. Peña 1110, 4, Buenos Aires, Argentina.

1st Vienna meeting of the International Association for the Promotion of Clinical and Experimental Research in Medicine molecular base of malignancy new clinical and therapeutic evidences, will be held in Vienna, Austria, Oct. 30–31 1974.

Organizing committee. E. Deutsch, K. Moser H. Rainer A. Stacher

Inquiries. Dr H. Rainer c o Wiener Medizinische Akademie, Alser Strasse 4 A 1090 Vienna, Austria.

Journées Médicales Annuelles de L'Hôpital Broussais – La Charité, service du professeur Paul Milliez, auront lieu 6–8 Mai, 1974 Journées consacrées aux acquisitions médicales récentes.

Il est recommandé de s'inscrire assez tôt, le nombre des participants étant limité. Prière d'envoyer les droits d'inscription au Centre de recherches sur l'hypertension artérielle, Professeur Milliez, Hôpital Broussais, 96 rue Didot, Paris-XIV France.

The 19th annual meeting of American Institute of Ultrasound in Medicine will be held at the Washington Plaza Hotel, Seattle, Washington USA, Oct. 6–9 1974.

Abstracts due July 1 1974 will be published in the Journal of Clinical Ultrasound and in the final program.

Address for all inquiries: AIUM 4 333 W Kinnear Palace Seattle, Washington 98119 USA.

The V World Congress of Gastroenterology organized by the Mexican Association of Gastroenterology with the support of the Organisation Mondiale de Gastro-entérologie, will be held in Mexico City Oct. 13–19 1974 The congress will solve around a program formed by conferences, symposia, informal discussions, study groups and scientific films.

Free paper sessions will be integrated with the papers accepted by the Scientific Program Commission. Complete texts of reports received in triplicate before April 1 1974 will be taken into consideration.

Further Information. V Congreso Mundial de Gastroenterologia, Avenida Veracruz 93 México 11 D F., Mexico.

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EDITORIAL

THE CORONARY CIRCULATION AND ACUTE MYOCARDIAL INFARCTION

The initiating events leading to acute necrosis of the myocardium are still largely unknown. In the last decades an *increased incidence of myocardial infarction* has been noted. However there seems to have been no increase in other manifestations of arteriosclerosis or in the extent of coronary vessel arteriosclerosis (1 17 18). This raises the question whether other mechanisms than gross changes of the coronary vessels, e.g. an increased vulnerability of the myocardium to ischemia (2), play a significant role in inducing an acute myocardial infarction (AMI).

Since Herrick's description of coronary thrombosis (16) it has been more or less universally thought that the coronary thrombus is a cause of myocardial necrosis. On the other hand, it is well recognized that a coronary thrombus is not a prerequisite for an AMI and is probably absent in most cases. When the infarction is of a transmural type the chance of finding a thrombus at autopsy is high, whereas it is seldom found in subendocardial infarction.

Several authors have criticized the theory that coronary thrombosis is a cause of AMI (6, 12, 20). As early as in 1939 Gross and Sternberg (13) proposed that the coronary thrombus might be a secondary event, and since then several reports have supported this notion. Spain and Bradess (21) found an increase of coronary thrombi at autopsy with the length of survival in patients with myocardial infarction and Branwood and Montgomery (5) and Emmrich (8) have found microscopic evidence that the coronary thrombi in some instances might be more recent than the corresponding myocardial necrosis. Hellström (15) induced myocardial infarction in the dog and found a coronary artery stasis, probably on the basis of vasospasm, that might be a contributory factor for the formation or growth of a coronary thrombus. The significance of the thrombus has also been questioned because it is commonly located at the site of a severe stenosis *where collaterals have had time to develop (3)*. Accordingly the complete occlusion of such a stenosis might have an insignificant effect on the coronary circulation. The speed of the occlusive process must, however, be taken into account since it is known from animal experiments that myocardial necrosis can be avoided by slow occlusion of a vessel (11).

Recently an attempt was made to further clarify the time relationship between coronary thrombosis and myocardial infarction (9). The authors studied the incorporation of [illegible]I-labelled fibrinogen into coronary arterial thrombi. In 6 patients, with ECG signs of myocardial injury suggesting start of necrosis, the fibrinogen was incorporated into the thrombus. In one of the patients who received fibrinogen 47 hours after the onset of symptoms only the end parts of the thrombus were radioactive whereas the major central portion was non-radioactive. Thrombus formation might thus still take place as late as 47 hours after the onset of a myocardial infarction. In the other 5 patients the entire thrombus was radioactive. However the technique used in this study cannot exclude the presence of a small non-radioactive portion, formed before the injection of fibrinogen in any of the thrombi. Such a small "primary" thrombus might well start the process of necrosis. On the other hand, the study favours the notion that the coronary thrombus in fact might be a secondary event in AMI.

Even if the coronary thrombus is a totally secondary event, its growth might have some effect on the local coronary circulation. The coronary arterial tree is not an end arterial system. A rich net of collaterals exists both epicardially (4) and via arteries of the B type from the subendocardial plexus

(10). Between an occlusion and the myocardial necrosis there is a non-infarcted region with an ischemic zone supported by these collaterals. Both a proximal and distal growth of the thrombus might occlude such collaterals and hamper the circulation of the ischemic zone distal to the occlusion and increase the infarction Furthermore, additional arteries of the A type might be occluded and also increase the infarcted area. Whether the growth of a thrombus has any significant effect on the ischemic zone or the size of the infarction is not known. No sensitive methods are yet available for deciding infarction size or for studying growth of a coronary thrombus in vivo.

Microcirculatory disturbances in AMI in man have been little studied. However recently a study with potential application to man was published. Moschos et al. (19) studied radioactively tagged platelets after inducing a coronary thrombus in the dog. The platelets were found to aggregate in the ischemic zone and, by treating the dogs with antiplatelet drugs, the ischemic zone was seen to decrease. Platelet aggregates may form at the site of an injury to the coronary vessel, e.g. an ulcerated plaque or rupture of the wall, or locally in the microcirculation. Platelet aggregates, however are but one factor that can occlude the microcirculation. Emboli of atheromatous debris or small thrombi may have the same effect. Platelet aggregates have been found mainly in patients dying suddenly (14) but how platelets behave in the microcirculation in AMI in man remains to be determined. Several other factors have been proposed (7) to be able to impair the microcirculation but their significance is even less known today Perhaps changes in the microcirculation will prove to be of greater importance than gross arterial lesions which have so far attracted most interest.

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HOSPITAL MORTALITY AFTER MITRAL VALVE REPLACEMENT

Prognostic Significance of Preoperative Clinical and Hemodynamic Factors

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Abstract. One hundred consecutive cases of mitral valve replacements have been retrospectively reviewed. The prognostic significance of preoperative clinical and hemodynamic factors for the early postoperative period has been evaluated. The hospital mortality was 21%. Of the patients above 60 years 42% died compared to 14% of the patients below this age ($p < 0.005$). The mean pulmonary arteriolar resistance was 446 dyn/sec cm^{-5} in the patients who died and 286 in the survivors ($p < 0.05$). The mortality was markedly increased, 62% in the patients with pulmonary arteriolar resistance above 600 dyn/sec cm^{-5} compared with the rest of the patients (p 0.0025). The other factors reviewed (functional class NYHA, heart volume measured roentgenologically pressure data, cardiac index, number of valves replaced and type of prostheses used) showed no statistically significant differences between the survivors and the early deaths. The main mode of death was found to be heart failure and sudden arrhythmia.

The early mortality after mitral valve replacement differs widely from one cardiovascular clinic to another. There is, however general agreement that in most patients a clinical and hemodynamic improvement is obtained *after successful operation* (2, 5 10). The preoperative clinical impressions of the physician and surgeon have been claimed to be the best parameter in the prognostic evaluation (4). The aim of our investigation was, through a retrospective study to correlate preoperative clinical and hemodynamic findings with mortality in the early postoperative period, in our study defined as the postoperative hospital period irrespective of its duration.

MATERIAL AND METHODS

One hundred consecutive patients, 63 women and 37 men, in whom mitral valve prosthesis was implanted between Jan. 1970 and June 1972 are included in the study. They had undergone clinical and roentgenological examination and were grouped in functional classes I–IV according to the criteria of the New York Heart Association (NYHA). The main clinical data are presented in Table I. The preoperative hemodynamic investigation of all patients included retrograde left heart catheterization with left ventricular angiography. Most patients were also subjected to right heart catheterization including pressure recordings, determination f cardiac output, using Fick principle, and calculation of pulmonary arteriolar resistance (PAR). Selection for surgery was made in joint meeting between cardiologist, surgeon and roentgenologist, discussing all relevant data. A rather liberal policy has been followed, as no absolute cardiologic contraindication to surgery has been defined. Practically all patients severely incapacitated by mitral valve disease were offered surgical treatment, irrespective of the duration or etiology of the disease. Eighty patients were given single mitral valve prosthesis, while 20 had either an aortic or tricuspid valve in addition. Lillehei, Beall and Björk–Shiley types of prostheses were used.

RESULTS AND DISCUSSION

The results are presented together with the clinical data in Table I. Early postoperative mortality was found to be 21% and this corresponds well with that of other centres, which report figures between 11 and 24% (1 3 7 8 9 11). It has been suggested that one reason for the high mortality might be that many of the patients were seriously ill (1 3). In our study the mortality in function classes II and III (NYHA) added together was 19% (13 pats.) compared to 24% (8 pats.) in class IV. The difference is not statistically significant. Thus we did not find that the class IV patients, as a group, had a more serious prognosis in the early postoperative period even though it contained many extremely ill patients,

Table I *Mitral valve prostheses implanted 1970–72 (30 months). Clinical hemodynamic and operative data*

		Total group	Survivors	Early deaths	
Total no. of pats.		100	79	21 (21%)	
Women		63	48	15 (24%)	
Men		37	31	6 (16%)	
Age (y)	Mean	53 (range 27–70)	52	58	
	> 60	24	14	10 (42%)	$p<0.005$
	< 60	76	65	11 (14%)	
Function class (NYHA)	II	11	10	1 (9%)	
	III	56	44	12 (21%)	n.s.
	IV	33	25	8 (24%)	
Heart rhythm, preoperatively	Sinus rhythm	16	11	5 (31%)	n.s.
	Atrial fibrillation	83	68	15 (18%)	n.s.
	Atrial tachycardia	1	0	1 (100%)	
Heart volume (ml/m² BSA)	Mean	791 ($n=91$)	786 ($n=72$)	792 ($n=19$)	n.s.
Intracardiac pressures (mmHg)	Right atrial (mean)	5.1 ($n=76$)	4.8 ($n=60$)	6.2 ($n=16$)	n.s.
	Pulm. art. (syst.)	52 ($n=71$)	50 ($n=56$)	58 ($n=15$)	n.s.
	Left ventric. (end-diast.)	8.5 ($n=95$)	8.5 ($n=75$)	8.5 ($n=20$)	n.s.
Pulmonary arteriolar resistance (dyn sec cm⁻⁵)	Mean	322 ($n=72$)	286 ($n=56$)	446 ($n=16$)	$p<0.05$
	< 600	64	53	11 (17%)	$p<0.0025$
	> 600	8	3	5 (62%)	
Cardiac index (l/min/m² BSA)	Mean	2.4 ($n=76$)	2.5 ($n=59$)	2.1 ($n=17$)	n.s.
Valves replaced	Single mitral	80	63	17 (21%)	n.s.
	Mitral + aortic	18	14	4 (22%)	
	Mitral + tricuspid	2	2	0 (0%)	
Valve, types of prostheses	Lillehei	55	44	11 (20%)	
	Beall	31	24	7 (23%)	n.s.
	Björk-Shiley	14	11	3 (21%)	

n.s. = not significant.

...ncy cases and reoperations for prosthesis failure

Ten of the patients older than 60 years (42%) died in the early postoperative period compared to 11 (14%) among those below this age. The difference is statistically significant ($p<0.005$) and is in agreement with other studies (4).

Left ventricular enlargement has been claimed to be a risk factor (4). We have not evaluated this parameter but we did not find any difference in heart volume measured roentgenologically between the survivors and the early deaths.

The mean left ventricular end-diastolic pressure was the same in both groups, while the mean right atrial pressure was somewhat higher among those who died. The difference was, however not statistically significant.

The patients who survived the early postoperative period had a mean resting cardiac index (CI) of 2.5 l/min/m² BSA compared to 2.1 among those who succumbed. There was, however a considerable scatter among the values and the difference was found not to be statistically significant. Five of the patients who died (31%) had preoperative sinus rhythm and 15 (18%) had atrial fibrillation. This difference is not statistically significant. One patient had preoperative atrial tachycardia and died of ventricular fibrillation. Retrospectively evaluated digitalis intoxication might have contributed to the arrhythmias in this case.

Increased PAR is a common finding in mitral valvular disease and is claimed to increase the operative risk (2). Barclay et al. (1) have found a strong correlation between pulmonary hypertension and both early and late postoperative death. They recommend that pulmonary hypertension with systolic pressure above 110 mmHg should be regarded as a contraindication to valve replacement. On the other hand, when the patient has successfully survived the postoperative period, several investigators (2, 5, 6, 11) find that

Table II. *Preoperative data and mode of death*

Pat. no.	Age (y.)	Functional class (NYHA)	Heart volume (ml/m² BSA)	PAR	Postoperative survival (d)	Mode of death
1	57	III	950	150	<1	Heart failure
2	64	IV	650	1 408	<1	Heart failure
3	61	IV	630	—	<1	Heart failure
4	61	III	670	650	1	Heart failure
5	56	III	1 315	—	1	Heart failure
6	53	IV	515	735	1	Heart failure
7	60	IV	1 040	426	2	Heart failure
8	64	III	690	1 040	3	Heart failure
9	54	III	670	926	3	Heart failure
10	56	II	980	—	4	Heart failure
11	64	III	925	—	12	Heart failure
12	67	III	—	230	77	Heart failure
13	44	III	730	110	104	Heart failure
14	61	III	940	239	1	Ventricular fibril'
15	60	IV	—	120	5	Ventricular fibrillation
16	55	III	530	390	5	Ventricular fibrillation
17	52	IV	875	333	<1	Cardiac arrest
18	59	III	700	215	3	Cardiac arrest
19	66	IV	850	130	4	Respiratory insufficiency
20	46	IV	670	26	20	Septicaemia
21	50	III	720	—	30	Valvular thrombos.

the pulmonary artery pressure decreases. This, however is no absolute rule as the pressure sometimes remains unchanged and may even rise (5 6).

In the present study the PAR among the patients who did not survive was significantly higher than that of the survivors. Among 8 patients who had a preoperative PAR above 600 dyn/sec cm⁻⁵ 5 died. This represents a mortality of 62% compared to 17% for the rest of the group. The difference is highly significant ($p < 0.0025$) and PAR seems to be the most important single factor in preoperative prognostic evaluation. We did not, however find any statistically significant difference in systolic pulmonary artery pressure between the survivors and those who succumbed.

An increasing operative mortality with increasing number of valves replaced is reported (3). Among our patients 20 had 2 artificial valves implanted at the same time, and the mortality in this group was the same as among 80 who had only one. This discrepancy may be due to the small number of patients in our study. On the other hand Starr (9) also reports the same mortality in double as in single valve replacement. Three types of valve prostheses were used in our study and there was no difference in per cent fatalities between the types.

The main mode of death was found to be intractable heart failure (13 pats. 62) despite optimal medical treatment (Table II). It is interesting to note that all patients with pre-operative PAR above 400 dyn/sec cm who died belonged to this group. Left ventricular diastolic pressure was, however normal in all these patients. In 5 patients the fatal outcome was due to sudden arrhythmia, in 3 ventricular fibrillation and in 2 cardiac arrest. Three of them died 3–5 days after the operation and this emphasizes the importance of continuous intensive care including ECG monitoring for several days. These findings correspond with earlier studies (3 7) where the most frequent fatal complication was found to be low cardiac output, while others (8) have found the most serious postoperative complication to be systemic embolism, uncontrollable arrhythmias and bacterial endocarditis. In the early postoperative period only one of our patients had a serious thrombotic complication and died from acute valvular thrombosis. Most of our patients were on anticoagulant therapy when operated upon, and in the others it was started immediately after the operation.

In conclusion we found that the early mortality after mitral valve replacement is relatively high. The main risk factors associated with significantly

increased mortality were age above 60 years and increased PAR. Among these factors increased PAR is the most important, and the mode of death in patients with a preoperative PAR above 400 dyn/sec cm^{-5} was intractable heart failure. The mortality was very high in patients with PAR above 600 dyn/sec cm^{-5} 62% although we admit that our figures in this group are small.

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CLINICAL AND MORPHOLOGICAL STUDIES OF GIANT HYPERTROPHIC GASTRITIS (MÉNÉTRIER'S DISEASE)

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Abstract. The authors describe four cases of giant hypertrophic gastritis (Ménétrier's disease), which demonstrate that the disease is easily overlooked clinically. In all four cases the definite diagnosis was established after morphological examination of the surgical specimens. The importance of gastroscopy, gastric secretory studies, studies of the protein metabolism and X-ray investigation of the upper gastrointestinal tract for the clinical diagnosis is emphasized, even if a definite diagnosis seems possible only after examination of full-thickness surgical biopsy specimens.

Giant hypertrophic gastritis, or Ménétrier's disease, is a rare gastric mucosal lesion involving the entire stomach or a part of it and is characterized by marked enlargement of the gastric mucosal folds. The etiology is unknown. The disease may be combined with adenomas of endocrine glands (11, 19). More recently autoimmunity has been discussed as a cause of Ménétrier's disease (7). The patients may suffer from dyspepsia or be free from symptoms, but as a result of protein loss in the stomach hypoproteinemia with edema may occur. Gastric secretory studies may show hypo- or achlorhydria.

In spite of the fact that the disease was described as early as 1888 (12), only about 200 cases have so far been reported in the literature. However, there are reasons to believe that the incidence of the disease is much higher than this figure indicates. With modern diagnostic methods, such as fibregastroscopy, gastrocamera and albumin turnover studies, Ménétrier's disease will probably be diagnosed clinically more often.

Concerning the clinical, morphological and differential diagnoses reference is made to the extensive review by Görsch (8). The frequent finding of hypoproteinemia in Ménétrier's disease can nowadays be studied by radioactive methods using, for example, I-131 albumin and I 131 polyvinyl pyrrolidone (2, 4, 9, 18). Increased quantities of albumin in the gastric juice can also be detected by paper electrophoresis or starch gel electrophoresis (4, 10).

The purpose of this paper is to draw attention to this rare disease, which in our experience may easily be overlooked, and to present four cases examined in the Institute of Pathology II, University of Gothenburg, between 1962 and 1968.

CLINICAL FINDINGS

Case 1

A 21-year-old man developed epigastric pains in 1960. The symptoms were relieved by food and antacids. He only remained symptom-free until 1962. Radiological studies of the upper gastrointestinal tract showed multiple filling defects involving the whole stomach except the antral region. Gastroscopy showed marked gastric folds. Gastric acid secretion was within normal limits (Table I). Biopsy from the body of the stomach showed mucoid metaplasia and a slight reduction of the parietal cell mass. Some cysts basally in the mucosa were observed. Interstitially there was a moderate infiltration of lymphocytes and eosinophilic leucocytes.

With the return of gastric pains the patient was readmitted in Jan. 1963. Vagotomy and pyloroplasty were performed. However, the gastric pains continued and in 1964 he also had hematemesis. Radiological examination of the upper gastrointestinal tract showed marked gastric folds in the whole stomach with hazelnut-sized filling defects. A Billroth II resection was performed. After the operation the patient improved rapidly. On reexamination in 1970 the patient reported that since the last operation he had had only slight epigastric pains during the spring and autumn but that he had not been off work. At this examination he had a normal Hb level of 16.. g/100 ml and a normal plasma protein level (protein 7.7 g/100 ml, albumin 4.8 g/100 ml).



Fig. 2. Case 3. The thickened mucosa with irregular hyper trophic folds with an appearance similar to the convolutions of the brain.

Regenerative gastric polyps differ morphologically from Ménétrier's disease in their charac teristic zonal structure and their more abundant



Fig. 3. Case 4. The thickened mucosa from the body of the stomach showing straight tubular glands, which are irregular and cystic basally and lined with metaplastic, mucoid epithelial cells. McManus × 30.

and edematous stroma. In addition the mucosa should theoretically be normal between the polyps in polyposis (6).

Not only in chronic hypertrophic gastritis of the interstitial, proliferative and glandular forms (15 16), but also in hypertrophic hypersecretory gastropathy (HHG) (17), the gastric glands may be normal or greatly increased in mass. The parietal and chief cells are not destroyed and replaced by metaplastic mucus-secreting cells as in our cases. However mixed forms of Ménétrier's disease, gastric polyposis and HHG may occur (3 17). It has been suggested that the classical Ménétrier's disease might represent a "burned out" phase of HHG (7).

Intestinal metaplasia with goblet cells, striated border and Paneth s cells can be seen in Ménétrier's disease but, as in our case 2, only in small areas. We agree with the opinion that intestinal metaplasia should not be considered an important feature in this disease (14). Progression and conversion of Ménétrier s disease to gastric atrophy with intestinal metaplasia have however been described (7).

Our patient 1 had a classical history of peptic ulcer but the augmented histamine test showed a normal output of acid (Table I). Radiologically polyps in the stomach were suspected. The diagnosis of Ménétrier's disease was first proposed after mucosal biopsy when mucoid metaplasia and cysts were seen, but a firm diagnosis was made only on the surgical specimen. This case

suggests that biopsy by gastroscopy does not allow a definite diagnosis of Ménétrier's disease. We agree with the opinion that it seems to be impossible to differentiate between this disease and different types of gastritis in a biopsy not comprising the whole gastric mucosa and the muscularis mucosae (16, 17).

In case 2 the difficulties of forming a correct clinical diagnosis were even greater. The clinical history, the X-ray findings and the presence of hypoproteinemia and edema were suggestive of the disease on the first admission two years before the operation. However, the diagnosis of Ménétrier's disease was not considered on that occasion. Later on, Ménétrier's disease was suspected on clinical grounds and albumin turnover was investigated with albumin-labelled I 131. The albumin disappearing rate was increased, which supported the diagnosis. The same was true with the augmented histamine test, which showed

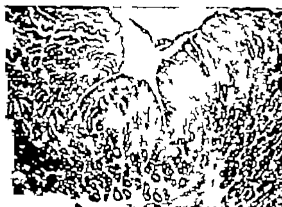


Fig. 5. Case 4. A polypoid protuberance with depressed centre. Note that the mucosa and muscularis mucosae are thinner in the depressed centre than at the periphery. Most of the dark tubular cells are mucoid metaplastic cells. McManus 22.

a reduced output of acid (Table I). The most prominent feature in the secretory pattern was the low acidity and the high volume both basally and maximally after histamine stimulation. The low acidity was probably due to an increased reabsorption of hydrogen ions in the damaged mucosa in exchange for sodium. This case illustrates the value of gastric secretory and albumin turnover studies in the diagnosis of Ménétrier's disease to differentiate this disease from HHG and Zollinger–Ellison's syndrome, which are characterized by extreme hypersecretion (1, 17, 19).

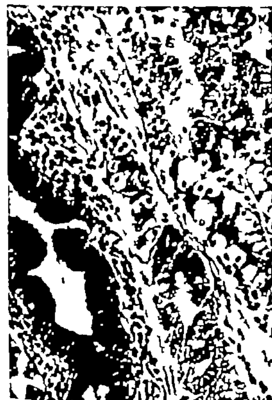


Fig. 4. Case 3. Tubular glands from the body of the stomach. The light parietal cells can easily be distinguished from the darker ones, which are mucoid metaplastic cells and chief cells. McManus 192.

In case 3 the clinical diagnosis of gastric malignancy could perhaps have been corrected by more careful investigation before surgery and total gastrectomy avoided. This case indicated that Ménétrier's disease may be difficult to differentiate radiologically from carcinoma or lymphosarcoma (4, 8).

Granulomas in the mucosa in Ménétrier's disease have been observed by others (7) and were found in our case 3. The cystically dilated gastric glands basally in the mucosa and submucosa in Ménétrier's disease seemed to retain their continuity with supraadjacent dilated glands. Where the granulomas were found, this continuity may have been broken and the outflow of the mucus been blocked.

In case 4 a Billroth I operation was performed when the patient was operated on for a ;

peptic ulcer The surgical specimen revealed a gastric ulcer besides the morphological appearance of Ménétrier's disease. This constitutes a rare case of the concomitant occurrence of these two diseases (cf 13), and it was in this case impossible to decide whether the symptoms emanated from the ulcer from the Ménétrier's disease or from both.

Initially as in case 1 or in mild cases of Ménétrier's disease conservative treatment with repeated small meals and antacids may relieve the symptoms of dyspepsia (7). However most cases such as ours are not diagnosed before marked symptoms and signs have appeared, such as severe pains, bleeding and edema with hypoproteinemia. In these cases partial or subtotal gastrectomy is sometimes necessary for relief from symptoms. The protein loss may also, as in case 2, be corrected by surgery (8, 9 12). However it is interesting that even spontaneous regression has been reported (7).

The possible connection between gastric carcinoma and Ménétrier's disease is not clear It has been recommended to make an intensive follow-up of patients treated conservatively because of the possible risk of malignancy (8). The occurrence of carcinoma in Ménétrier's disease, however is probably coincidental (14) and no cause ffect relationship has ever been proved" (5). operative specimens in our cases showed y no epithelial atypias suggestive of arcinoma, and the follow-up of the present patients has not revealed any clinical signs of malignancy

The present investigation has attempted to draw attention to the clinical diagnosis in Ménétrier's disease and also to describe the morphology of the stomach in this disease. Clinical investigations including gastroscopy with biopsy gastric secretory studies, studies of the protein metabolism and X-ray investigations of the upper gastrointestinal tract are important for the diagnosis. However as in the four cases described, it appears that a firm diagnosis of Ménétrier's disease can only be established definitely after morphological examination of the surgical specimen.

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PROGNOSIS AFTER FIRST MYOCARDIAL INFARCTION

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Abstract. The study includes 404 patients with their first myocardial infarction, admitted to Coronary Care Unit within 24 hours of the onset of symptoms. Sixty-two patients (15%) died during the first month following admission. A follow-up examination was carried out of the remaining patients after minimum observation time of 12 months and maximum of 38 months. It was possible to demonstrate, using the life-table method, that patients with their first myocardial infarction have significantly poorer survival rate than the normal population. In patients still living one month after the acute attack of first myocardial infarction it was shown: 1) that there is poorer survival rate with increasing age, 2) that women have poorer survival rate than men, partly because the average age of the women is higher and partly because the old women (> 70 years) have considerably higher mortality rate than the old men, 3) that the site of the infarction, the magnitude and duration of the ST elevation in standard ECG leads in the acute phase are of no prognostic importance for long-term survival.

It would be desirable that those patients who survive the acute phase of a myocardial infarction and who belong to a high risk group could be identified before discharge from the hospital, so that the subsequent treatment could be planned with this in mind.

Several parameters have been presented that give a poorer long-term survival, e.g. previous myocardial infarction, heart failure, diabetes mellitus and hypertension, and a number of complications during the acute myocardial infarction (AMI) such as shock, hypotension, congestive heart failure and certain arrhythmias (2, 5, 6). Kibe and Nilsson (9) also found that the long-term prognosis was related to maximum enzyme activity (SGOT, LDH), but this could not be confirmed in a recent study (6).

As recent studies (10–12) have shown that ST segment elevation gives important information concerning the risk of death in the acute phase, it was decided to study the possibility of using these initial ECG changes also to identify patients with a particularly great risk of death in the further course of the disease. In addition to this a study has been made of the influence of age and sex on the long-term prognosis.

MATERIAL AND METHODS

The study includes 305 men and 99 women who had been admitted to the Coronary Care Unit of the University Hospital of Odense from Nov. 1969 to Dec. 197. All of these patients had AMI according to the following definitions: precordial pain of long duration and/or acute pulmonary edema in connection with the occurrence of signs of infarction in ECG and/or characteristic increase in enzymes. In addition the following criteria were fulfilled by all patients: 1) first myocardial infarction, 2) admission to the Unit within 24 hours of the onset of symptoms, 3) at least one ECG with 12 leads and at least one series of serum enzyme determinations (SGOT, LDH and CPK) prior to cardiac arrest or death.

Grouping of the patients

Of the total of 404 patients included in the study 62 died during the acute phase (days 0 to 30 inclusive). The remaining 342 were divided into four groups according to the site of the infarction (evaluated according to the ECG) and the magnitude of the ST elevation.

1) Anterior infarction (126 patients). Cases with ST elevation ≥ 5.0 mm in one or more leads (I, AVL and V_1–V_6) were placed in the group "major ST elevation" (62 patients), the remainder in the group "minor ST elevation" (64 patients).

2) Inferior infarction (91 patients). Cases with ST elevation ≥ 2.0 mm in one or more leads (II, III and AVF) were placed in the group "major ST elevation" (46 patients), the remainder in the group with "minor ST elevation" (45 patients).

3) Multiple infarctions (56 patients). Cases with ST elevation ≥ 3.0 mm in one or more leads (I, AVL, V_1–V_6) and ST elevation ≥ 2.0 mm in one or more leads (II, III, AVF) were placed in the group "major ST elevation" (19 patients), the remainder in the group "minor ST elevation" (37 patients).

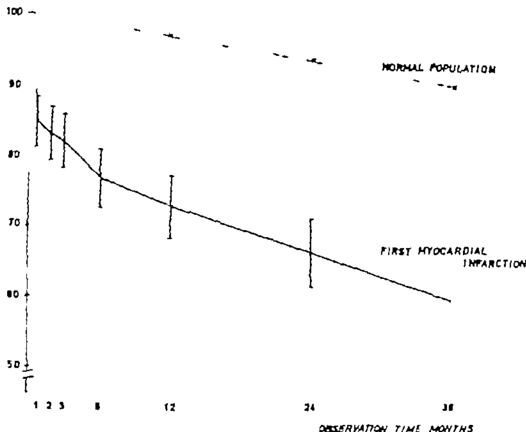


Fig 1 Survival curve of patients with first myocardial compared to that of matched normal population

4) Infarctions of uncertain localization together with cases in which the QRS complex was increased in width (≥0.1 sec) and BBB (69 patients). Six patients with strictly posterior infarction are also included in this group

The definition of major and minor ST elevation was such that groups of almost equal size were obtained. Of those patients who survived, 127 had major and 146 minor ST elevation.

The same number of patients were found in the subgroups following division according to the duration of the ST segment elevation: 1) short duration with ST elevation for <2 weeks (146 patients), 2) long duration with ST elevation for >2 weeks (127 patients).

Definition during measurement of the ST elevation

An ECG was taken daily during the first week, using the following leads: I, II, III, AVR, AVL, AVF V_1–V_6. In the following 3 weeks the ECG was usually taken 2 3 times per week, or as required.

The elevation of the ST segment on the ECG was measured by using the TP segment as the isoelectric line. When the S wave was present, the deviation was measured 0.06 sec after the nadir in mm to the nearest 0.5 mm.

In some patients without an S wave, measurement was started 0.06 sec after the nadir of the QS complex or of the Q wave. In very few patients without well defined S, QS or Q waves the R wave was used as the starting point. These definitions did not permit measurement of the ST elevation in patients with a broad QRS complex (0.12 sec or more), and for this reason these patients are included in the group of infarctions of uncertain localization.

Follow-up

The patients have been followed-up on Jan. 1, 1973 The minimum period of observation was 12 months, the maximum 38 months. The information regarding the patients was obtained from hospital records, National Registration Offices, general practitioners, Medical Officers of Health, The Central Register of Deaths of the National Health Service.

Of the 404 patients 401 were traced. The remaining 3 were foreigners, who were visiting this country All 3 were discharged alive.

Statistical analysis

The life-table method has been employed in the calculation of the survival probability (3, 4). Confidence limits of

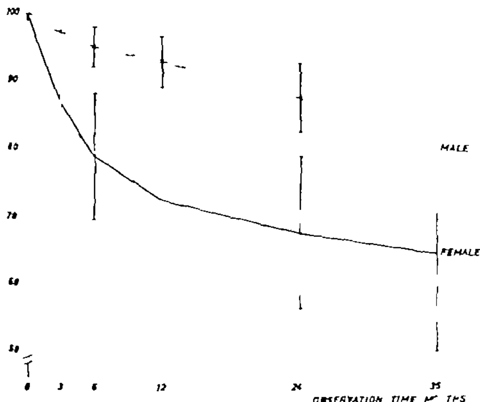


Fig. 2. Relative survival curves of patients still living one month after the acute attack of first myocardial infarction, according to sex.

95% have been used in the survival curves. The standard deviation has been calculated with the help of "Greenwood" estimate (4). Statistical comparison between the survival rates has been carried out partly by evaluation of the curves with safety intervals and partly using the 2 $n\chi^2$ test, where is the number of observation intervals (1).

Comparability

The frequency of risk factors (diabetes mellitus, hypertension and hyperlipidemia) together with complicating diseases (chronic pulmonary and renal disease) in patients still living one month after the first myocardial infarction was compared in all the subgroups. A significant difference occurred only once: this will be referred to under Results.

RESULTS

The observed survival rate for the patients in the study are shown in Fig. 1. Each patient has been matched with a person of the same age and sex in the Danish population. From the death rate tables based on the registrations of deaths in Denmark in the years 1969–70 (18), a survival curve was drawn which represented the expected survival rate in a normal population. There is a statistically significant difference between the survival curves. Forty-two men and 20 women, i.e. 15% of the patients, died during the first month of observation (days 0 to 30), which is considered as the acute phase of the disease—a definition which is justified by the course of the curve. The women had a tendency to higher mortality than men in the acute phase but the difference was not significant ($\chi^2 = 2.380$). After an observation time of one year it can be seen that the annual mortality is around 6.5%.

The 3-year survival rate in males and females surviving one month (day 30) after the acute attack of first myocardial infarction is shown in Fig. 2. The relative survival is the relationship between the observed survival in the material and the expected survival in a matched normal population. The relative survival is considered to be

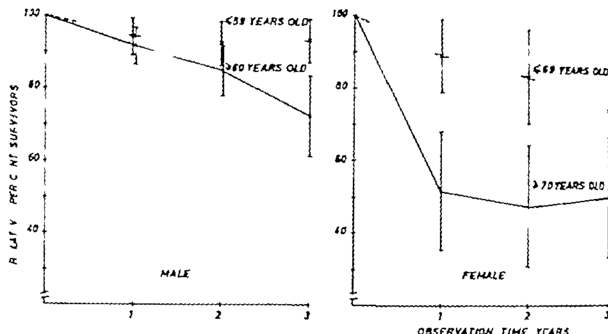


Fig. 3. Relative survival curves of patients still living one month after the acute attack of first myocardial infarction, according to sex and age.

the survival rate in a theoretical population in which myocardial infarction is the only cause of death (3). It can be seen that females have a lower survival rate than males. The difference significant from 3 months to 2 years after the phase. The average age for men was 60.9 for women 69.6 years.

Fig. 3 shows that patients recovering from the acute phase of myocardial infarction have a decreased relative survival with advancing age. This applies both to men, men 59 years old or less having a better survival rate—significantly better after 3 years—than men 60 years or more, and to women, women 69 years of age or less having a better survival rate than women of 70 years or more. The difference is significant in the first 2 years.

Fig. 4 shows the relative survival curves for males and females 70 years of age or more surviving one month after the acute attack. Women have, after three months, a significantly lower survival rate than men. It can be seen that the curves descend during the first 24 months and thereafter ascend, in other words the life-threatening influence of the myocardial infarction remains with the old patients for 2 years after the acute phase. Ninety-one patients were 70 years or above, and of these patients 18 were 80 years or above. The latter group of patients consisted of 10 women, of whom 4 died during the observation time, and 8 men, of whom 3 died.

There were 107 men and 27 women aged between 60 and 69 years who survived the acute phase. There was no significant difference in survival rate between the two sexes in this age group.

Of the previously mentioned 91 patients of 70 years or more 54 were men and 37 women, of whom 13 men and 21 women died. Autopsy was performed on 21 of these (62%). The causes of death could be divided into three groups, based on the findings of the autopsy: 1) cardial (reinfarction, cardiac failure): 5 men and 9 women, 2) thromboembolic complications, 1 man and 4 women, 3) non-cardiovascular diseases, 1 man and 1 woman. There was no significant difference between the causes of death of men and women. Eleven of those not subjected to autopsy died suddenly in their homes (5 men and 6 women), while information on the mode of death was not given in 2 cases. The cause of death was stated to be cardial on the death certificates of all 13.

The 3-year survival rate is shown in Table I.

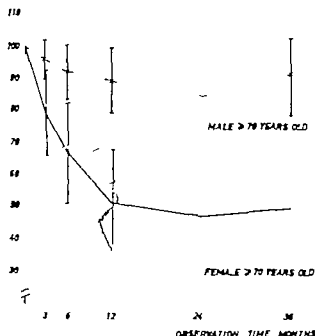


Fig. 4. Relative survival curves of patients, 70 years and over still living one month after the acute attack of first myocardial infarction, according to sex.

for the patients who survived one month after the acute attack, divided into subgroups according to the site of the infarction. There was no significant difference in the survival rate for the four types of infarction, either with regard to men or women.

Table II shows the 3-year survival rate for patients who survived one month after the first myocardial infarction, with respectively major and minor ST elevation in the ECG in the acute phase. There was no statistically significant difference between the survival rates in the two groups, either for men or women.

The 3-year survival rate for patients who survived one month after the acute attack, and who had short and long duration, respectively of ST segment elevation in the ECG in the acute phase is shown in Table III. Significantly more risk factors and complicating diseases were found in the male patients with short duration of ST

Table I. *Life-tables of patients still living one month after first myocardial infarction, according to localization of infarction*

x = observation time in years, lx = alive at beginning of interval, dx = died during interval, Fx = effective number exposed to the risk of dying, $Fx = lx - Ux + Wx/2$, where Ux are untraceable during interval and Wx are withdrawn alive during interval (Ux and Wx are not included in the present tables), Px = cumulative proportions surviving through interval

	Anterior				Inferior				Multiple				Uncertain			
	lx	dx	Fx	Px	lx	dx	Fx	Px	lx	dx	Fx	Px	lx	dx	Fx	Px
Males																
0–1	101	7	99	0.93	76	5	73	0.93	39	6	39	0.85	47	8	46	0.83
1–2	90	7	69.5	0.84	69	4	53.5	0.87	33	3	26.5	0.75	37	1	27.5	0.80
2–3	42	4	23	0.69	38	1	20	0.82	17	1	9.5	0.67	17	1	9	0.71
Females																
0–1	25	6	25	0.76	15	4	15	0.73	17	6	17	0.65	22	8	21	0.62
1–2	19	1	14.5	0.71	11	0	7.5	0.73	11	1	8.5	0.57	12	2	8.5	0.47
2–3	9	0	5	0.71	4	1	2.5	0.44	5	0	2.5	0.57	3	0	1.5	0.47

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CARDIAC ARREST FOLLOWING ACUTE MYOCARDIAL INFARCTION

A Study of 285 Cases from Three Medical Departments Using a Joint Acute Admission Section Containing a Coronary Care Unit

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Abstract. During 38-month period 1 108 cases of acute myocardial infarction have been treated in three medical departments ith joint acute medical admission section containing coronary care unit (CCU) 285 of them had cardiac arrest (26%). In 209 cases (73% of cardiac arrests) resuscitation was attempted, in 66 cases (23%) ith primary success. Twenty-five patients (9%) could be discharged from hospital Two to four years later 17 ere still alive, 14 in heart function class I, 12 with unchanged working bility Cardiopulmonary resuscitation later than 24 hours after admission to the CCU improved the rate of survival after myocardial infarction by less than 0.5%. I this study the survival after cardiac arrest is somewhat lower than usually reported from CCUs. This is supposed to be due to our treatment of less selected patient group, and possibly to the untraditional integration of CCU in an acute medical admission unit. This may lead to a less intensive treatment of the cardiac patients, while it is considered an advantage for the non-cardiac acute medical patients.

Following the establishment of the coronary care units (CCU) a number of centers have reported reduced hospital mortality after acute myocardial infarction (AMI). The main reason for this is thought to be the prevention of arrhythmias leading to cardiac arrest, and a more efficient treatment of actual cardiac arrest. The optimal length of each patient's stay in the unit is, however still being discussed, and recently the value of the CCUs has been questioned from an economic as well as from a therapeutic point of view As the role of the CCU in the prevention of death from myocardial infarction is not finally settled, we present the results of treatment of cardiac arrest in a group of patients with myocardial infarction from three medical departments with a joint acute admission section containing a CCU

METHODS AND MATERIAL

The acute dmission section has been described in det l elsewhere (8). The section has 22 beds, 8 of hich ha equipment for ECG monitoring of patients with myocardi infarction. Patients with uncomplicated myocardial infarctio were to be discharged from the CCU after 6 days, and stayed another 3 eeks in the ordinary medical ards without any special observation. The number of available beds in the CCU has, however proved to be too small. In number of cases the stay in monitored bed as shortened day or two, and one third of all patients with AMI could not be treated in the CCU at all. In general, younger patients ith severe myocardial infarction had the highest priority for the monitored beds.

The diagnosis of AMI as based on the finding of any two of the following criteria: history ECG changes, and elevation of serum lactate dehydrogenase. In most of the fatal cases the diagnosis as verified by autopsy

Cardiac arrest was defined as an abrupt cessation of the effective circulation, due to arrhythmias or sudden cardiac collapse, and requiring resuscitation. Sudden unexpected cardiac death was also considered cardiac arrest even if resuscitation was not attempted. In these cases non-cardiac cause of death was excluded by autopsy

Patients who were dying in cardiogenic shock, in advanced heart failure or had severe non-cardiac diseases at the time of their cardiac arrest were not included in the group of cardiac arrests, although resuscitation was tried in some of these cases. Patients who ere dead on arrival at the hospital ere also excluded.

Resuscitation was considered primarily successful if the patient survived for at least 12 hours with intact respiration, circulation and cerebral activity

Using these criteria we have reviewed the records of all patients with AMI treated in the medical departments of Bispebjerg Hospital, Copenhagen, in the 38-month period Oct. 19 1967 Dec. 31 1970. In Dec 1972 the patients still surviving cardiac arrest were interviewed and examined.

I the statistical treatment of the results we hav used the χ^2-test and Fisher's exact test, choosing the 0 05 level as our level of significance.

Table I. *Factors of possible prognostic significance related to the incidence and mortality from cardiac arrest among 1108 cases of AMI*

	Incidence of factor (%)		Death from cardiac arrest (%)	
	Patients without cardiac arrest ($n=823$)	Patients with cardiac arrest (–285)	Patients without factor	Patients with factor
Previous infarction	20	24	21	23
Hypertension	8	9	21	21
Diabetes	9	12	21	28
Congestive heart failure on admission	18	18	21	22
Pulmonary edema after admission	12	13	21	22

RESULTS

Of 1108 cases with AMI 285 (26%) were complicated by cardiac arrest. In 209 cases (73%) resuscitation was attempted, with primary success in 66 cases (32% of the attempted resuscitations and 23% of all cardiac arrests) Twenty-five patients (9% of all cardiac arrests) survived for one month and 17 (6% of all cases of cardiac arrest) for more than two years.

Table I shows that previous infarction, hypertension and diabetes demanding treatment before admission, congestive heart failure on admission (i.e. peripheral edemas or pulmonary stasis) and pulmo- [illegible] edema during the hospitalization carried a [illegible] greater risk of cardiac arrest. In no case, [illegible] was the difference statistically significant. Neither were the differences between the mortalities from cardiac arrest statistically significant. The arrhythmias leading to cardiac arrest are not recorded in all our cases, and accordingly they can provide no prognostic information in this study

The age of the patients and the outcome of cardiac arrest are seen in Table II. The occurrence of cardiac arrest was unrelated to age, and the higher mortality among older patients was correlated to fewer attempts at resuscitation rather than to the response to the resuscitation.

Table III shows the location of the patients at the time of the cardiac arrest. The survival was much better when the cardiac arrest occurred in the CCU where resuscitation was attempted in almost all cases. The actual outcome of resuscitations was the same in the three locations, but the attempts are fewer outside the CCU

The time of cardiac arrest is seen in Table IV Late in the course of the myocardial infarction resuscitation was attempted in fewer cases, but the primary response to resuscitation was similar to that of the early cases.

In Dec. 1972, 2–4 years after the myocardial infarction and cardiac arrest, 17 patients were still alive. Tables II and V show their age, heart function and working ability Fourteen patients showed an

Table II. *Age related to incidence and outcome of cardiac arrest in 285 patients among 1108 cases of AMI*

CA = cardiac arrest, RES = resuscitation

		CA		RES attempted		RES primarily successful			Survival for 1 mo.		Survival for 2 y	
Age (y)	No. of AMI	No.	% of AMI	No.	% of CA	No.	% of CA	% of RES	No.	% of CA	No.	% of CA
<50	56	14	25	13	93	9	66	70	6	43	3	21
50–59	181	40	22	36	90	13	33	36	6	15	3	8
60–69	335	99	29	80	81	24	25	30	8	8	8	8
70–79	377	101	27	69	68	17	17	25	5	5	3	3
>80	159	31	20	11	35	3	10	27	0	0	0	0

Table III. *Location during cardiac arrest and outcome of cardiac arrest in 269 patients*

16 patients who had cardiac arrest outside these locations (e.g. in ambulance, emergency room) are excluded.
CA cardiac arrest, RES – resuscitation

Location	No. of pats.	RES attempted No.	RES attempted %	RES primarily successful No.	RES primarily successful % of CA	RES primarily successful % of RES	Survival for 1 mo. No.	Survival for 1 mo. % of CA
CCU	148	142	96	41	28	29	19	13
Acute admission ward outside CCU	42	27	64	6	14	22	3	7
Medical ward	79	28	35	9	11	31	1	1

increase in body weight of 2–20 kg (mean 7 kg). The chest X-ray in all cases showed an enlarged heart, only in four increased since the discharge. Only one patient had been controlled at regular intervals by cardiologists at the hospital since the discharge, and four of the remaining 16 had felt the want of such a control program.

Eight patients had survived one month after the myocardial infarction, but had died before the follow-up interview. In two cases the cause of death was a new cardiac arrest, in two a new myocardial infarction, and in four congestive heart failure.

DISCUSSION

Resuscitation was attempted in only 73% of the cases in this group of patients with cardiac arrest. Most untreated cardiac arrests occurred in the ordinary medical ward, half of them late in the course of a myocardial infarction (i.e. at a time and place where the cardiac patients are under no special observation). The establishment of the acute admission section and CCU has tended to make the ordinary medical ward a quiet place, where emergencies seldom happen, and where the attention of the nursing staff has turned towards the nursing of chronically ill patients. As compared to the time before the establishment of the CCU (and other intensive care units), the mortality after myocardial infarction may even have increased in ordinary medical wards at the same time as it is decreasing in the CCU (2, 5). The late sudden deaths after myocardial infarction are probably caused by the same arrhythmias as the cardiac arrests treated in the CCUs, and death could possibly be prevented if the arrhythmias were discovered in time for treatment.

If we were to consider the hypothetical question of what the survival might have been if we had used another routine observation time in the CCU we find that very small changes would occur. Using the death rate for patients having cardiac arrest in the CCU on all patients with cardiac arrest, the result is an increase in the number of survivors of not more than 10 patients. So, even by extending the observation time in the CCU to the whole hospital stay the increase in survivors would be less than 1% of all our patients with myocardial infarction.

Conversely if in a similar calculation we suppose that all cardiac arrests during the first 24 hours in hospital occurred in the CCU and all later cardiac

Table IV *Time and outcome of cardiac arrest in 285 patients*

CA = cardiac arrest, RES = resuscitation

Day after infarction	CA No.	CA % of all CA	RES attempted No.	RES attempted % of CA in each group	RES primarily successful No.	RES primarily successful % of CA	RES primarily successful % of RES	Survival for 1 mo. No.	Survival for 1 mo. % of CA in each group
1	123	43	104	84	34	28	33	12	10
2–7	89	31	66	76	22	25	33	11	12
8–31	73	26	37	50	10	14	27	2	3

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Table V *Conditions of 17 patients surviving cardiac arrest for 2–4 years*

Heart function class[a]	Total no. of pats.	Unchanged working ability	In active work
I	8	6	4
II	6	5	2
III–IV	3	1	1

[a] According to the New York Heart Association.

arrests in the ordinary medical wards, the number of survivors would be reduced by four patients, less than 0.5% of all our patients. It seems possible to reduce the stay in a monitored bed to 24 hours with hardly any notable change in mortality after cardiac arrest. Of course these considerations do not account for the possible change in the frequency of cardiac arrest due to the preventive antiarrhythmic treatment.

The death rate after cardiac arrest in our study does not compare too favourably with the 55–84% reported by other hospitals with CCUs (4 6, 10, 11 12, 14 18, 20). These materials seldom include the patients who die suddenly without attempt at resuscitation, and often resuscitation is more or less reserved for patients under 70 years of age. The for the high death rates in this study is, how- only the age of the patients or the number cardiac arrests. The mortality among -scitated patients under 70 is as high as 84%. We have no proof that our patients are in a worse cardiac condition on admission than those of other hospitals, although this has sometimes been our impression. Comparisons with other materials on this point are extremely difficult on account of the highly varying and often non-specified diagnostic criteria. The frequency of cardiac arrest in this study is not greater than that reported by other CCUs, and complicating conditions (Table I) do not seem to change the prognosis. A contributory reason for the relatively high death rate may be our integration of the CCU in an admission section, serving three medical departments. No doubt this involves the arrival of more critically ill patients because of the shorter transportation time. The system in itself may tend to make the treatment of patients in the CCU less efficient, at the same time as the treatment of most other acute medical patients is becoming more efficient. For each patient with myocardial infarction the admission section receives 14–16 other acute medical cases, all being treated by the same nursing staff as the coronary patients, and by doctors from three medical departments. As a result the training and experience of each nurse and doctor in treating coronary patients may be less than that of a completely specialized unit with its own staff. Our coronary care system may not be optimal, and a reorganization is being planned. Recently cost-benefit analyses have raised doubt about the value of the CCUs (1), and their advantage in the treatment of pulmonary edema has been questionned (9). We are quite convinced that the CCU can prevent some deaths from cardiac arrest, and possibly even prevent some cardiac arrests. It is, however evident in this study that treatment of cardiac arrest after the first day or two can reduce our hospital mortality less than 1% of the number of patients with myocardial infarction.

Discussing possible ways of improving the survival after myocardial infarction, it should be remembered that only about half of all patients with this disease survive long enough to reach hospital (3 15). We should think that some of the energy an money spent on resuscitations might find a better use in attempts at earlier diagnosis and treatment of myocardial infarction, maybe even in the prevention of the disease (13).

The long-term survival after cardiac arrest is quite the same as in other reports (7 16, 17), and not different from the long-term survival for all patients with myocardial infarction (9 19). An unexpected finding was the increase in weight without corresponding clinical edema or pulmonary congestion. Although part of the weight gain may be explained by some accumulation of interstitial fluid, we feel that a reduced physical activity after the myocardial infarction is the major reason. An information and control program with regular follow-up examinations is needed for these patients.

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THE EFFECT OF SOYA LECITHIN ON SERUM LIPID VALUES IN TYPE II HYPERLIPOPROTEINEMIA

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Abstract. A group of 12 patients with type II hyperlipoproteinemia has been treated orally with a soya lecithin preparation. After an initial control period of 4 weeks 1.2 g soya lecithin was given per day. This amount was doubled after 4 months for an additional period of 4 months. The investigation was concluded with second control period of 4 months. The following lipid parameters were determined. total lipids, total cholesterol, triglycerides, phospholipids, total lipids in the lipoprotein fractions, and the weight percentage of linoleic acid in serum cholesterol esters and the serum lecithin. No clinically relevant changes in these parameters were observed.

The establishment of elevated blood cholesterol levels as a risk factor in ischaemic heart disease has created a worldwide drive for measures by which serum cholesterol levels can be lowered.

The use of lecithins high in linoleic acid content has been reported to cause a decrease in serum cholesterol in many cases. Administration may be either intravenously (13) or orally (9 17)· results are achieved more rapidly by the i.v. method. A high content of polyunsaturated fatty acids is claimed to be an important requirement for successful results with the lecithin (2). An attractive feature of the use of polyunsaturated lecithin is that the daily amounts required are small and compare favorably with those of polyunsaturated triglycerides.

Whether or not the results of lecithin treatment depend on the type of patients is still largely a matter of speculation. In previous studies the patients were not clearly defined, for instance in terms of the Fredrickson types of hyperlipoproteinemias (8).

The present study was therefore undertaken to investigate the effect of the oral administration of lecithin on the cholesterol level (and on the level of other lipids) in the serum of 12 clear-cut patients of the Fredrickson type II.

MATERIAL AND PROCEDURE

The group studied consisted of 12 patients, 3 males 9 females, aged 34–76 years, with Fredrickson type II hyperlipoproteinemia. Typing was established by determining lipids, total cholesterol and triglycerides by chemical m.t and on the basis of lipoprotein analyses performed combination of ultracentrifugation and precipitation applying the criteria described in previous communicat. (16, 18).

Relevant data concerning the typing of the patient are summarized in Table I. The data for the lipoprotein composition in percentages by weight of total lipids, the serum cholesterol and serum triglyceride values are given as obtained during control period of 4–5 weeks preceding the period of treatment. All patients, except no. 4 have percentage of total lipids in the β-lipoproteins of over 60 % and ratio of cholesterol to triglycerides > 3 4, phenotyping them as type II hyperlipoproteinemia. In patient 4 no floating

Table I. *Lipid parameters during 1st control period*

Chylo = chylomicrons
Cholesterol and triglyceride values are the means of 4 determinations on different samples except for patients 1 and 4 in whom the figures are the means of 5 determinations

Pat. no.	Total lipids in the lipoprotein fractions ( ) Chylo	Pre-β	β		Serum cholesterol (mmol/l)	Serum triglycerides (mmol/l)
1	2	14	63	71	8.91	1.5
2	1	14	67	18	8 75	1.5
3	1	8	71	20	8.87	0 6
4	2	35	49	14	10.65	3 4
5	2	13	60	25	8.42	1.8
6	4	16	62	18	7.90	1.3
7	3	6	76	15	13.00	1 1
8	1	18	72	9	1[illegible].75	[illegible]9
9	1	16	65	18	8.75	1.8
10	1	12	76	11	14 10	1.4
11	3	21	63	13	17 40	3.3
12	1	5	76	18	10.16	1.0

Table II. *Data on the patients*

Pat. no.	Height (m)	Weight (kg) Before	After	Age (y)	Sex	Diagnosis[a]	Drugs[b]	Diet[c]
1	1 77	72	70	42	♂	Hyp. M. inf.	AH Diu, Se	Normal
2	1.63	66.5	65	58	♀	AP Hyp., M. inf.	AH Diu, VD	Normal
3	1.59	54	55.5	34	♀	No complaints	None	Normal
4	1.59	61	49.5	50	♀	AP M. inf.	AC, Dig, Se, VD	Chol. low
5	1.47	52	55	76	♀	AP M. inf.	Diu, Se, VD	Chol. low
6	1.55	63.5	68	48	♀	AP M. inf.	VD	Chol. low
7	1.56	68	69	66	♀	AP	Diu, Se, VD	Normal
8	1.53	59	58.5	63	♀	Hyp., VS	AC, AH, Dig, VD	Chol. low
9	1.71	56.5	55	43	♂	AP	AC, BB Dig, Se, VD	Normal
10	1.83	70	65	57	♂	AP MI	VD	Normal
11	1.58	66	62	44	♀	M. inf	AC	Normal
12	1 63	85	89.5	45	♀	No complaints	None	Normal

AP = angina pectoris, Hyp. = hypertension, M. inf. = myocardial infarction (occurring at least 6 months previously), MI mitral insufficiency VS = vascular sclerosis.
AC = anticoagulant, AH = antihypertensive, BB = β-blocker Dig = digoxin, Diu = diuretic, Se = sedative, VD = vasodilatator
Chol. low = cholesterol lowering.

β-lipoproteins could be detected, therefore, this patient was also regarded as belonging to type II (IIb).

T ble II gives relevant data on the patients. Ten were out patients f the Department of Cardiology of the Wilhelmina Hospital, Amsterdam, who were under control for angina pectoris or atherosclerotic lesions. The hyperlipidemia of the other two patients (nos. 3 and 12) was discovered during a family medical examination. These two patients had no complaints. All had normal fasting blood sugar values (<5.5 mmol/l) and normal protein-bound iodine value (<0.7 μmol/l). Antibodies against thyroid tissue were absent in all

he diet which was followed before the beginning of the gation was adhered to as strictly as possible during the and the patients were checked at regular intervals to make sure that they adhered to the diet. Eight were on regular Dutch diet and four on cholesterol-lowering diet (Table II).

Weight alterations during the study were moderate, except in one patient (no. 4) who lost 11 5 kg (Table II). She was for several months in the mental ward of the hospital during the investigation and had complaints of anorexia.

Any medication prescribed with the aim of correcting the hyperlipidemia was stopped some time before the beginning of the investigation. Other medications were continued unchanged during the whole observation period.

Before starting the treatment with lecithin, blood was taken 4 times (in two patients 5 times) at intervals of one week to establish the starting level of the serum lipids (1st control period). After this initial control period the patients were given 4 capsules (1.2 g) soya lecithin day for period of 10–20 weeks. The preparation used was "lecithine essentiale—forte—Natterman—Versuchspräparat 5180" and each capsule contained EPL (soya lecithin), 300 mg; vitamin B_1, 6 mg; vitamin B_6, 6 mg; vitamin B_{12}, 6 μg; nicotinamide, 33 mg; and vitamin E-acetate, 6 mg. During this period (1st experimental period) blood was drawn once every two weeks.

From measurements on several relevant food components, it became clear that the amounts of essential phospholipids ingested daily by way of the consumed food were within the range of 200–400 mg. After the first experimental period had lasted for approximately 5 months, it became evident that no clear-cut effect of the drug on serum lipids was detectable. Therefore it was decided to raise the dose of lecithin to 2 4 g (8 capsules) day for an additional period of 4 months. Blood samples were taken once every three weeks during this 2nd experimental period. The investigation was concluded with a 2nd control period of 4 months during which blood was taken once month.

METHODS

Total lipids, total cholesterol, and triglycerides were determined in all serum samples at the Wilhelmina Hospital by the following methods.

Total lipids were measured by the colorimetric method of Chabrol and Charonnat (5). Pooled serum of known total lipid content as determined by extraction and weighing according to Folch et al. (7) as modified by Prins et al. (16) was used as standard. The extraction procedure as described by Abell et al. (1) was used for total cholesterol, and for triglycerides the method of van Handel and Zilversmit (14) was employed. Electrophoresis of lipoproteins, done during the first control period, was performed on cellulose acetate as described previously (18). In addition the following lipid parameters were determined at the Gaubius Institute: Phospholipids were measured by means of combination of densitometry on thin layers of silica gel and lipid phosphorus determination (10). Lipoproteins were fractionated by combination of ultracentrifugation and precipitation (11) into the following fractions: Chylomicrons, very low density (pre-β), low density (β), and high density (α) lipoproteins. The amount of lipids in these fractions was determined by weighing after extraction with methanol-chloroform sub-

Table III. *Influence of soya lecithin on serum cholesterol levels (mmol/l) during the different periods (means ± S.E.M.)*

Pat. no.	1st control period (n)		1st experimental period (n)		2nd experimental period (n)		2nd control period ( )	
1	5	8.91 ± 0.41	12	8.31 ± 0.34	5	8.80 ± 0.28	4	9.60 ± 0.36
2	4	8.75 ± 0.57	9	8.19 ± 0.21	5	8.42 ± 0 28	4	8.70 ± 0.44
3	4	8.87 ± 0 08	5	8.40 ± 0.10	6	8.50 ± 0.34	4	8.87 ± 0.36
4	5	10 65 ± 0.31	8	10.24 ± 0 44	4	9.06 ± 0.49	4	9.06 ± 0 41
5	4	8.42 ± 0.08	14	8.42 ± 0.10	6	8.90 ± 0.23	3	9 15 ± 0.10
6	4	7.90 ± 0.52	12	7.21 ± 0.18	6	7.29 ± 0.28	1	7 86
7	4	15 00 ± 0 26	12	14.10 ± 0.36	5	16 08 ± 0.41	4	16.10 ± 0 39
8	4	12.75 ± 0.80	5	14.22 ± 0.23	6	14 78 ± 0.44	4	14.90 ± 0.49
9	4	8.75 ± 0.36	13	8.50 ± 0.26	6	8.80 ± 0.34	4	8.42 ± 0.10
10	4	14.10 ± 0.98	13	13.38 ± 0 31	6	13.38 ± 0.47	4	13.92 ± 0.36
11	4	17 40 ± 0.52	13	16.58 ± 0.31	6	17.20 ± 0 83	4	17 70 ± 0.44
12	4	10.16 ± 0.31	5	10 42 ± 0.16	5	10 24 ± 0.36	4	11.60 ± 0.65
Mean ± S.E.M.		11.00 ± 0.91		10.64 ± 0 88		10.92 ± 0 98		11.32 ± 1.01

1st experimental period vs. 1st control period $p = 0.1$
2nd experimental period vs. 1st control period $p = 0.9$
2nd control period vs. 1st control period $p = 0.25$.
2nd experimental period vs. 2nd control period $p = 0.01$

ture (11). The lipoprotein composition is given as percentage of total lipids (Table I) or as g/l (Table IV). The fatty acid composition of the serum sterol esters and the serum lecithins was determined by gas-liquid chromatography after isolation of the esters by thin-layer chromatography and methylation.

The lecithins of the food products were analysed after extraction of total lipids with methanol-chloroform and weighing followed by phosphorus determinations in total lipids and in isolated lecithins. Fatty acid determinations in lecithins were accomplished as mentioned above.

RESULTS

Table III shows the mean serum cholesterol values during the two control and the two experimental periods as determined by the colorimetric method. The p-values, calculated by use of the Student's t-test on individual differences, show that the small differences between the two experimental periods and the first control period are not statistically significant. There is, however, a significant statistical difference between the second control and the second experimental period, but this difference is very small and has no clinical relevance. Table IV shows the concentration of lipids in the β-lipoproteins, which is a measure of the amount of cholesterol in these lipoproteins. The only statistically significant difference is again between the second experimental period and the second control period but, in this case also, the difference is too small to have clinical relevance.

Table V summarizes the mean values for the total lipids, triglycerides and phospholipids during the indicated periods. Only one of of the 12 calculated p-values gives a significant difference, the one for the difference in total lipids between the second experimental period and the second control period.

Table IV *Influence of soya lecithin on the concentration of lipids in β-lipoproteins (g/l)*

Pat. no.	1st control period	1st exper. period	2nd exper period	2nd control period
1	6.3	6 3	—	5 4
2	7 1	5.3	5.8	5.3
3	6.3	5.6	5 7	6 1
4	6.6	5.7	5.5	6 2
5	6.1	6.1	5 5	6 7
6	5 8	5 0	5 0	—
7	10.1	11 5	11.3	11.0
8	10.4	10.9	10 0	10 7
9	7 1	5 7	5.8	6 0
10	10 3	9.8	9.0	9.8
11	11.9	12.5	12.8	13.7
12	7 7	7.5	6 9	8.6

t-test on individual differences.

	$\bar{d}$	p
1st experimental period vs. 1st control period	−0.31	0 2.
2nd experimental period vs. 1st control period	−0.33	>0 05<0.1
2nd control period vs. 1st control period	−0.03	0.9.
2nd experimental period vs. 2nd control period	−0.58	>0 01<0.02.

$\bar{d}$ is the mean of the individual d

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INCREASE IN PLASMA α-LIPOPROTEINS IN CHRONIC ALCOHOLICS AFTER ACUTE ABUSE

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Abstract. The plasma α-lipoprotein levels have been measured in chronic alcoholics admitted to an alcohol clinic. The α-lipoprotein concentration was found to be increased in 60 of 69 patients after recent drinking bouts. The values were as rule normalized within two weeks. The increase in α-lipoproteins was not correlated with other plasma lipoprotein or lipid alterations, nor was any correlation found between high -lipoproteins and liver damage detected by analyses of bilirubin, SGOT SGPT GT and bromsulphalein retention tests.

An abnormal metabolism of lipids and lipoproteins is not uncommon in alcoholism. The plasma triglyceride level is raised and sometimes strikingly high (1 12, 16, 23) and lipoprotein electrophoresis reveals hyperlipoproteinemia similar to types I, IV or V according to the classification system of Fredrickson et al. (5).

Another obvious change in the plasma lipoprotein pattern, slight to moderate increase in the -lipoproteins, was recently reported by Johansson and Laurell (11). This change was detected on agarose electrophoresis of plasma proteins from alcoholics and its nature was revealed with immunochemical methods. The present study was undertaken in order to assess the frequency of this lipoprotein change and the effect of withdrawal of alcohol on the α-lipoprotein levels. The α-lipoprotein disorder was studied for any correlation with other plasma lipoprotein and lipid changes as well as with liver damage.

MATERIAL

The clinical material consisted of male in-patients of the Alcohol Clinic, Malmö General Hospital. The patients' ages ranged from 28 to 59 years. All of them were advanced γ-alcoholics according to Jellinek's classification system (7) and had used alcoholic beverages for on the average, 18 years (range 6–35). Many were in poor physical condition and often intoxicated on admission. Their last bout had lasted for 5 days to about 2 months, during which their diet had usually been very inadequate. Some patients had for various reasons been sober for several days before admission.

The normal range of α and β-lipoprotein was estimated in plasma samples from 30 apparently healthy men (aged 40–60 years) attending health control service.

Venous blood samples were obtained in the fasting state. Na_4 EDTA (disodium ethylenediaminetetraacetate) was used as anticoagulant to obtain plasma for lipoprotein and lipid analyses. All other analyses were performed on serum.

METHODS

Lipoprotein and lipid analyses

Lipoprotein electrophoresis was performed in agarose gel as described by Johansson (9). α- and β-lipoproteins were determined by electroimmuno assay (13). The antisera used were raised in rabbits immunized ith α- or β-lipoprotein prepared by preparative ultracentrifugation of serum, as described by Havel et al. (6). The antisera were rendered specific by absorption with ultracentrifugal fractions not containing the corresponding antigen.

Total plasma cholesterol as determined mainly in the way described by Ness (17), and plasma triglycerides according to Laurell (14).

Other analytical methods

Total serum bilirubin was determined in Technicon Auto-Analyzer using modification of the method of Jendrassik and Grof (18).

Aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) activities were determined at 35°C in an LKB reaction rate analyzer 8600 (LKB, Bromma, Sweden). γ-glutamyltransferase (GT) activities were measured with slight modification of the method of Orlowski and Meister (19).

Bromsulphalein retention was estimated as the percentage retained 30 min after injection of 5 mg dye/kg b.wt.

Plasma proteins (albumin, haptoglobin, immunoglobulins G and A) were determined with electroimmuno assay (13), supplemented by visual inspection of the electrophoretic pattern in agarose gel.

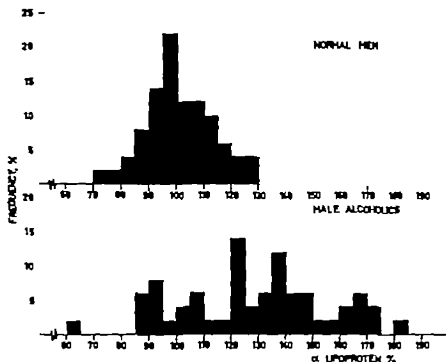


Fig 1 Distribution of α-lipoprotein in apparently healthy men and in consecutive male chronic alcoholics admitted to the hospital (percentage of the normal mean value for men).

RESULTS

Fig. 1 compares the distribution of immunochemically determined α-lipoprotein levels in 50 healthy males with that of 50 consecutive male in-patients of the Alcohol Clinic. It is clear from the comparison that α-lipoprotein was increased in half of e patients. This increase could often be recognized by visual inspection of the electrophoretic plasma lipoprotein patterns, in which the α-lipoprotein zone was relatively intense (Fig. 2).

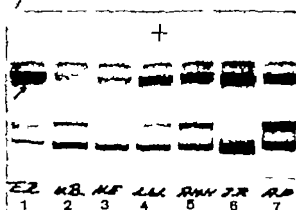


Fig 2. Agarose gel electrophoretic patterns of lipoproteins in plasma from male alcoholics with various α-lipoprotein levels. The α-lipoprotein zones are indicated by an arrow. The following α-lipoprotein values were found by immunochemical analysis (percentage of normal mean value for men): (1) 220%, (2) 128%, (3) 138%, (4) 160 %, (5) 165%, (6) 182%, (7) 170%.

Since not all these unselected patients were examined on the day of admission, a further study was undertaken on 95 patients in which all individuals were examined on the morning after admission. They were divided into three groups. Group I consisted of 69 patients who were intoxicated on arrival at the hospital or had been drinking until the day before admission. Group II consisted of 10 patients who had been sober for 3–4 days before admission, and group III (16 patients) of those who had not been drinking alcohol during the last week before hospitalization. Fig. 3 shows that α-lipoprotein was increased in all but 9 patients in group I—in two by more than 100%. In group III α-lipoprotein levels were invariably normal, while in group II they were increased in about half of the patients.

No pronounced changes in β-lipoprotein were found, though they were possibly somewhat low in group I. No correlation was found between the and β-lipoprotein levels in any of the groups. The triglyceride levels were increased in 13 out of 47 patients in group I (Fig. 3). But only one of the patients had severe hypertriglyceridemia in whom the α-lipoprotein level was 156% of the normal mean value for men. No correlation was found between



Fig. 3. Plasma levels of α-lipoprotein (*α-LIPO*), β-lipoprotein (*β-LIPO*), triglycerides (*TG*), and cholesterol (*CHOL*) in chronic alcoholics (percentage of normal mean values for men). Normal ranges are indicated. ○ = samples which were not analyzed for β-lipoprotein, triglycerides or cholesterol.

the triglyceride and α-lipoprotein levels. In 13 patients the α-lipoprotein was followed during their stay in hospital. In 10 of these patients the levels became normal within two weeks (Fig. 4). In two, who had the highest values on admission, after about three weeks (Fig. 4).

The liver function studies (Fig. 5) showed a slight hyperbilirubinemia in 7 cases, whereas a mild to moderate increase in the amino transferase activities was more common. GT activity was raised in most of the cases studied. The rise was moderate, except in one case in whom it was pronounced. That patient had mild bilirubinemia and moderately increased alkaline phosphate activity possibly suggesting biliary obstruction. Bromsulphalein retention tests, performed only in about 50% of the cases, showed increased retention in less than one third.

Plasma protein analyses showed signs of inflammatory reaction in 55% of the cases, but rarely any signs of involvement of the liver. The haptoglobin was low in five patients and the IgA level moderately raised in eight. The albumin levels were within normal limits. The plasma protein patterns thus showed no signs of advanced liver cirrhosis.

No correlation was found between the -lipoprotein increase and the results of liver function tests (cf. Fig. 6, which shows no correlation between α-lipoprotein and SGPT). Furthermore, the α-lipoprotein levels on the interruption of alcohol abuse

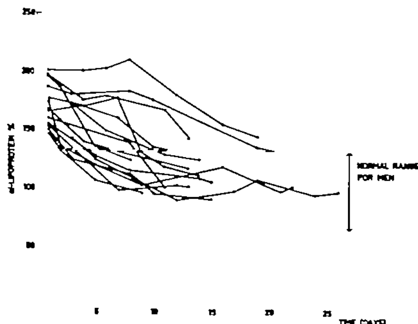


Fig. 4. Rates of normalization of α-lipoprotein in 13 patients after admission (percentage of normal mean values for men).

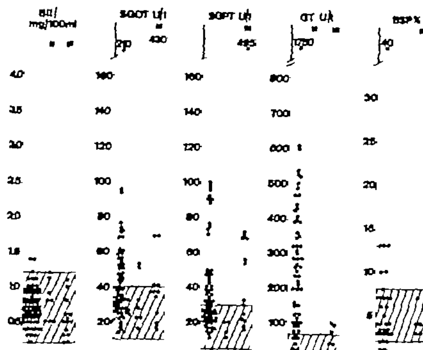


Fig 5 Values for serum bilirubin (Bil,t), SGOT SGPT GT and bromsulphalein retention (BSP) in chronic alcoholics. Normal ranges are indicated.

in ten patients studied were not attended by corresponding changes in the GT activities.

DISCUSSION

The findings suggested that increased plasma α-lipoprotein levels are common in chronic alcoholics. The material consisted of only male alcoholics, but such an increase occurs also in female alcoholics, if due regard is taken to the normally higher levels of α-lipoprotein in women (8, 22).

The rapid normalization of the α-lipoprotein on hospitalization and withdrawal of the alcohol suggests that the metabolic disturbance is of direct effect of a recent drinking bout rather than a chronic abuse. Factors other than the alcohol intake per se might also be thought to play a role in the rise in α-lipoprotein concentration, e.g. poor diet and a poor general physical condition, predisposing to respiratory and other infections, often reflected by the changes in the plasma protein patterns. But the inflammatory reaction cannot explain the rise in the α-lipoproteins, since it has been shown that such conditions are accompanied by lowering of α-lipoprotein levels (2, 10). The dietary factors cannot be ignored, but it should be noted that some of the patients with the high α-lipoprotein levels had a satisfactory diet. Furthermore, a slight but significant increase in plasma α-lipoprotein was observed in an experimental study in which healthy students on a normal diet were given 60 g ethanol a day for five weeks (3).

The cause of the α-lipoprotein increase is as yet unsolved. Preliminary studies have shown no significant difference between α-lipoprotein in alcoholics and normal lipoprotein with regard to lipid composition and molecular size distribution on Sephadex G 200 gel filtration, whereas the apoprotein composition remains to be established. It is quite evident that the increase can occur without concomitant increase of enzymes and bromsulphalein retention indicating liver damage. Even the few cases with normal GT values, known to be a most sensitive indicator of alcoholic liver damage (20, 21), had high α-lipoprotein levels. On the other hand, the presence of a fatty liver cannot be revealed by analysis of serum.

It is possible that the α-lipoprotein increase is in some way coupled to the altered metabolism of triglycerides and very low density lipoprotein elicited by the abuse of alcohol. The recent suggestion that at least part of the high density lipoprotein or α-lipoprotein is derived from very low density lipoprotein by lipolytic processes in plasma (15) supports such an assumption. More detailed studies are necessary to elucidate the exact interrelationship between these two classes of lipoproteins.

From a clinical point of view the α-lipoprotein

Fig. 4. Correlation between α-lipoprotein levels and SGPT in 43 chronic alcoholics. The upper limits are indicated by horizontal and vertical lines.

increase observed might prove useful in the diagnosis of a heavy consumption of alcohol. It should be noted, however that similar increases in α-lipoproteins were found in men exposed to chlorinated hydrocarbon pesticides (4).

ACKNOWLEDGEMENTS

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THE INTRAVENOUS FAT TOLERANCE TEST IN SUBJECTS WITH MASSIVE OBESITY

A Study before and after Jejuno-Ileal Shunt Operation

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Abstract. The i.v. fat tolerance test (IVFTT), in which the elimination rate of the fat emulsion Intralipid® is determined, has been performed in 60 patients with massive obesity. These patients were compared with a control group of matched subjects of normal weight with the same plasma TG concentrations. The hyperbolic correlations which were found between the plasma TG concentration and the IVFTT value did not differ between the two groups. The IVFTT value was correlated rather to the plasma TG value than to total body weight. The IVFTT values were higher in women of both groups. Twenty-three obese patients were studied with IVFTT before and after jejuno-ileal bypass operation. Concomitant with a mean weight reduction from 129 to 104 kg, plasma TG was significantly reduced from 1.92 to 1.40 mmol/l. The IVFTT value rose significantly from 3.99 to 5.83%/min. However in the individual cases the pattern was highly variable, as some patients lost weight considerably without major effects on the IVFTT value, whereas others had a marked fall of plasma TG and rise of IVFTT value concomitant with a very moderate weight loss. The results suggest that the reduced IVFTT values in massive obesity are of the same order as seen in primary hyperlipidaemia and that the removal rates for the Intralipid® emulsion are not proportionate to the amount of adipose tissue. Other factors than weight loss, such as changes of blood flow in the splanchnic region, could possibly account for the increased IVFTT values after jejuno-ileal bypass operations.

In the i.v. fat tolerance test (IVFTT) (8, 9) the elimination rate of the fat emulsion Intralipid® (Vitrum, Stockholm, Sweden) is followed after a single i.v. injection. The elimination of the emulsion follows first order kinetics below a so-called critical concentration and thus gives a straight line in a semilogarithmic plot. The elimination rate of Intralipid® expressed as the rate constant k_2 (%/min), is related to endogenous plasma triglycerides (TG) in several ways. The fractional turnover of endogenous plasma TG (4, 5) is well correlated to the k_2 values, suggesting that the k_2 values reflect the fractional removal rates of endogenous TG (21). Furthermore k_2 is correlated to plasma TG concentration in a hyperbolic way, subjects with low removal rates having high plasma TG concentrations and vice versa (8, 22). Several factors are known to affect the IVFTT value. In dogs noradrenaline has been found to cause a decrease of the fractional removal k_2 (12), and in this species glucagon also reduces the k_2 value significantly (13). In man several lipid lowering drugs, such as nicotinic acid, clofibrate analogues and oxandrolone, concomitantly with a reduction of plasma TG, have increased k_2 (6, 7, 17). Prolonged starvation in combination with a surgical trauma increases the k_2 value in man, and such an increase may also be seen after an acute myocardial infarction (11, 20).

The IVFTT value thus seems to be influenced by several hormonal and metabolic factors. It has been pointed out that one reason for these observed differences in k_2 may relate to the fact that the removal sites are not evenly distributed in the organism (10). For this reason blood flow changes secondary to hormonal and metabolic factors may make more or less of these sites available. These flow changes may either promote or counteract the effect of a hormone or metabolic product on the elimination process at the vessel wall. The role of the adipose tissue in the removal of the Intralipid® emulsion is not known in detail. In the carbohydrate-fed rat Olivecrona and

Table I *Clinical data, plasma lipids and IVFTT of obese subjects and age and plasma-TG-matched controls (mean ± S.E.M., ranges within parentheses)*

		Age (yr.)	TG (mmol/l)	Chol. (mg/100 ml)	Weight (kg)	Index weight (kg)/ height (cm–100)	k_2 (%/min)
Controls		42 ± 2	2.38 ± 0.28	277 ± 20	80 ± 2	1.01 ± 0.01	3.01 ± 0.31
Males	11	(36–49)	(0.99–3.76)	(203–396)	(67–90)	(0.94–1.07)	(1.56–4.96)
		41 ± 2	1.76 ± 0.13	261 ± 10	59 ± 2	0.93 ± 0.02	5.51 ± 0.32
Females	49	(21–62)	(0.65 ± –4.67)	(153–393)	(46–71)	(0.75–1.15)	(2.11–11.74)
Patients		41 ± 2	2.94 ± 0.30	219 ± 10	134 ± 11	1.70 ± 0.04	2.99 ± 0.27
Males	11	(27–52)	(1.02–3.78)	(189–280)	(122–160)	(1.49–2.00)	(1.89–5.30)
		38 ± 2	1.87 ± 0.11	229 ± 6	127 ± 3	1.90 ± 0.04	4.43 ± 0.25
Females	49	(18–63)	(0.72–3.80)	(122–362)	(94–176)	(1.41–2.75)	(1.20–9.11)

Belfrage (16) have demonstrated that chyle TG is removed from blood in two main ways. One part of the chyle TG is stored in the liver as intact particles and the rest is incorporated into tissue esters as TG fatty acids. The adipose tissue plays a quantitatively important role in the metabolism during the removal of the chyle TG. The adipose tissue is rich in lipoprotein lipase the enzyme necessary for the removal of plasma TG (19). In advanced obesity the fat cell diameter is generally increased (2, 26) and large fat cells have been shown to be metabolically more active and to have an increased TG turnover (1, 26).

The present study was undertaken to see … subjects with marked obesity eliminated … pid® otherwise than subjects with a more normal amount of adipose tissue. The IVFTT was therefore carried out in highly overweight patients and compared with non-obese controls. In some of the obese patients a jejuno-ileal shunt operation was made. After the operation the IVFTT was repeated at different intervals during the induced weight-reducing period caused by the postoperative malabsorption.

MATERIAL AND METHODS

Subjects

The obese patients studied had all been referred to the Department of Surgery, Karolinska Hospital, because of advanced obesity. They had over [illegible] years duration of their overweight, unsuccessful results of conservative medical treatment and social and/or psychiatric complications of their obesity. During the preoperative evaluation programme 60 patients were studied with the IVFTT. The clinical data of these patients are summarized in Table I. After operation the IVFTT was repeated in 23 patients (2 men, 21 women) 1–3 times during the period of weight reduction. The test was repeated 2–18 months after the operation (Table II). The bypass operation was in 9 patients an end-to-side anastomosis between jejunum and ileum and in 14 an end-to-end anastomosis (18, 23). The patients did not receive any instructions to change their diet after the operation. A multivitamin preparation (Protovit® Roche, Basel, Switzerland) was given daily and 1 mg B_{12} (Hepagon Novum® Astra, Södertälje, Sweden) was administered i.m. every two months. In some cases serum electrolytes had to be corrected with potassium orally and magnesium i.m. Diarrhoea was controlled by diphenoxylate chloride (Retardin® Leo, Helsingborg, Sweden).

Controls. In order to compare the preoperative IVFTT of obese patients with values from subjects of more normal weight, data from a control group were obtained. The control subjects were selected from earlier studies on the IVFTT (22). For every obese subject a matched control of the same sex, of approximately the same age and plasma TG concentration, was chosen. The controls were not on any drug or diet affecting lipid metabolism and had fairly normal body weights (Table I). The

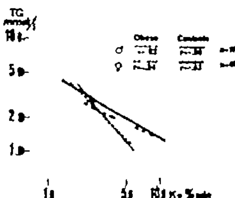


Fig. 1. Relationship between the i.v. fat tolerance and plasma TG concentration in obese patients and matched controls. All equations are significant ($p < 0.01$ for obese males, $p < 0.001$ for the other groups). Logarithmic scales.

Table II. *Clinical data and weight reduction pattern in obese patients before and after jejuno-ileal shunt operation*

					Postoperative study					
			Preop. IVFTT		1st IVFTT		2nd IVFTT		3rd IVFTT	
Subj. no.	Age (y.)	Sex	Height (cm)	Weight (kg)	Months postop.	Weight (kg)	Months postop.	Weight (kg)	Months postop.	Weight (kg)
1	55	♀	167	156	2	146	9	111		
2	46	♂	173	138	7	110				
3	50	♀	160	142	10	113				
4	47	♀	170	146	3	117	5	100		
5	31	♀	162	94	4	79				
6	43	♀	172	160	5	151	18	134		
7	45	♀	170	108	4	104	7	100		
8	24	♀	160	150	2	132	12	105		
9	18	♀	169	131	3	120				
10	27	♀	155	101	8	86				
11	32	♀	158	111	9	84				
12	45	♀	172	115	7	92				
13	34	♀	164	129	5	107	8	94	13	87
14	33	♀	176	137	2	119				
15	28	♀	174	115	2	110				
16	47	♀	163	134	10	106				
17	18	♀	178	143	14	90				
18	24	♀	174	111	16	83				
19	60	♀	170	130	11	111				
20	48	♀	162	158	6	122				
21	37	♂	180	160	3	149				
22	62	♀	151	110	3	94				
23	53	♀	171	100	2	96				

matched controls did not differ from the group they were selected from with regard to the IVFTT values and plasma lipids.

Analytical methods

All blood samples were taken after an overnight fast by venipuncture. Plasma TG and cholesterol were determined by AutoAnalyzer methods (3, 15). The IVFTT has recently been described in detail (9). A blank blood sample was taken and then 1 ml 10% Intralipid®/kg b wt. was given intravenously as single dose injection. Every 5 min 3 ml blood was taken for 40 min. The samples were centrifuged twice and plasma was diluted 1 : 100 in saline. The samples were read in Thorp micronephelometer. After subtraction of the blank the light scattering indices of the samples were plotted in semilogarithmic system, where straight line was found. The slope of the line was calculated by the method of least squares, and the elimination rate was expressed as %/min. Plasma volume was determined using labelled [125]I-albumin directly after the preoperative IVFTT (24). Plasma TG turnover rate was arbitrarily estimated as the product of k_2 and the plasma TG concentration.

RESULTS

Preoperative values

Plasma lipids. No significant difference of age and plasma lipids was found between male and female obese subjects and their corresponding control groups (Table I). Plasma TG were significantly higher in the obese males than in the obese females ($p < 0.001$). No significant differences of plasma cholesterol between groups and sexes were found. The male controls had a weight/height index of 1.01 ± 0.01 (S.E.M.) and the females 0.93 ± 0.02. The corresponding values in the obese group were 1.70 ± 0.04 and 1.90 ± 0.04.

IVFTT. In each group IVFTT was higher in females than in males. Control females had a 81% higher mean value, which was highly significant. In the obese group females had 48% higher IVFTT values than males, but this difference did not reach statistical significance.

Plasma TG turnover rate. The relationship between IVFTT and plasma TG for the obese patients and the control subjects is given in Fig. 1. All regression lines were significant. There is no significant difference between the slopes. If the product $k_2 \times TG$ mmol/l min was calculated in the groups, the value obtained for male controls was 6.43 ± 0.43 (S.E.M.) and for obese males 6.51 ± 0.45 mmol/l min. For female controls the corresponding values were 8.51 ± 0.50 mmol/l min

Table III *IVFTT body weight and plasma lipids in obese subjects during weight reduction before and after jejuno-ileal shunt operation*

	Preoperatively				Postoperatively 1st IVFTT			
Subj. no.	k_2 (%/min)	Weight (kg)	TG (mmol/l)	Chol. (mg/100 ml)	k_2 (%/min)	Weight (kg)	TG (mmol/l)	Chol. (mg/100 ml)
1	5.90	156	0.99	193	7.23	146	0.69	127
2	1.89	138	3.78	222	2.86	110	1.47	146
3	3.85	142	2.07	242	2.96	113	1.92	161
4	2.14	146	1.82	220	2.07	117	2.20	160
5	3.91	94	2.58	237	6.78	79	1.24	182
6	2.60	160	1.33	194	4.06	151	1.32	190
7	8.01	108	1.08	255	6.69	104	1.64	168
8	3.31	150	1.70	170	4.57	132	1.31	128
9	4.12	131	0.81	187	4.28	120	1.30	169
10	5.61	101	1.23	177	5.18	86	2.22	163
11	6.35	111	1.91	218	12.32	84	1.07	166
12	4.63	116	1.40	168	6.14	92	1.30	110
13	6.08	119	1.62	249	5.57	107	1.39	131
14	2.14	137	2.28	252	2.99	119	1.43	158
15	1.20	115	2.03	230	2.43	110	1.23	112
16	3.28	130	2.11	217	2.63	106	1.83	143
17	4.52	143	1.02	187	10.36	80	0.67	88
18	4.14	111	1.94	207	5.79	83	0.97	111
19	4.60	130	1.86	196	4.47	111	1.65	172
20	3.07	153	3.80	238	3.26	122	2.40	140
21	2.80	160	1.94	208	4.08	149	1.30	128
22	4.28	110	1.81	267	7.41	94	1.59	133
23	3.42	100	3.13	232	4.07	96	1.50	—

and for obese females 7.57 ± 0.38 mmol/l min. There were no significant differences between products.

asma volume (PV) was determined preopera ly in 14 obese patients. Turnover of plasma TG determined as $k_2 \times \text{TG} \times \text{PV}$ mmol/min did not correlate to body weight or the weight/height index. Plasma TG clearance determined as $k_2 \times \text{PV}$ l/min did not correlate significantly to weight/height index or body weight.

Table IV *Weight, plasma TG and cholesterol and the i.v. fat tolerance during the weight reduction period after jejuno-ileal shunt operation (mean ± S.E.M.)*

	Weight (kg)	TG (mmol/l)	Chol. (mg/100 ml)	k_2 (%/min)
Before operation	129 ± 4	1.92 ± 0.17	216 ± 6	3.99 ± 0.34
During weight reduction	104 ± 4	1.40 ± 0.09	145 ± 5	5.83 ± 0.67
n	23	23	22	23
p[a]	< 0.001	< 0.01	< 0.001	< 0.01

[a] Calculated on the individual differences.

Postoperative values

Twenty-three obese patients were studied with IVFTT repeatedly after the jejuno-ileal shunt operation. Table II gives the weight reduction pattern and weight at the time of the studies. In the patients who were studied more than once after operation the weight reduction rate expressed as kg b.wt./month is reduced with time. In Table III individual plasma TG cholesterol and the IVFTT values taken during the weight reduction period are given. The mean values of the measurements are summarized in Table IV. In this Table the values before operation have been compared with the results after weight reduction. When this latter study was performed some patients had reached a steady weight, whereas others were still losing weight. Concomitantly with a mean weight reduction from 129 to 104 kg there is a significant reduction of plasma TG from 1.92 to 1.40 mmol/l and an increase of the IVFTT from 3.99 to 5.83%/min, which is also significant. Plasma cholesterol concentration was significantly reduced from 216 to 145 mg/100 ml.

2nd IVFTT				3rd IVFTT			
k_2 (%/min)	Weight (kg)	TG (mmol/l)	Chol. (mg/100 ml)	k_2 (%/min)	Weight (kg)	TG (mmol/l)	Chol. (mg/100 ml)
13.97	111	0.87	145	8.16	92	0.78	127
4.81	100	1.68	174				
4.94	134	1.15	149				
7.21	100	1.01	173				
4.46	105	1.62	127				
6.77	94	2.02	192	10.79	87	0.90	145

The relationship between k_2 and plasma TG concentration during the weight reduction period is illustrated in Fig. 2, where the changes of k_2 and plasma TG are given in three groups with different body weight reduction patterns. In the operated subjects the weight reduction rate can be expressed as kg/month. This measurement did not correlate to the observed changes of k during the corresponding time.

DISCUSSION

When the regression lines for obese males and females, plotting log k_2 against log TG are compared to the control subjects, there is no significant difference between the slopes (Fig. 1). This finding suggests that the removal sites for the fat emulsion are not increased in proportion to the amount of adipose tissue. The development of obesity seems to have increased the plasma TG pool but if the number of removal sites does not increase the final result is a rise of plasma TG. The k_2 value after jejuno-ileal shunt operation is improved concomitantly with a fall of plasma TG and this finding could indicate that an improved elimination in the periphery rather than a change of plasma TG secretion rate at least partly may account for the reduced TG concentration in plasma.

The results suggest that reduced k_2 levels in marked obesity are of the same order as is seen in primary hyperlipidaemia (22). The fact that the weight loss after operation did not seem to be related to k changes also supports the concept that removal sites for the Intralipid® emulsion are not proportionate to the amount of adipose tissue.

The pattern in Fig. 2 is in principle the same as may be seen in other situations where plasma TG has been lowered by for instance, specific drugs (6, 7 17). Concomitantly with a plasma TG reduction an increase of k_2 is seen. Although the multiplication of k by plasma TG does not take heterogeneity of plasma TG with different lipoprotein turnovers into account (25), the product can serve as a model and yield some information about the observed changes. If turnover rate is expressed as $k_2 \times$ TG min, hanges

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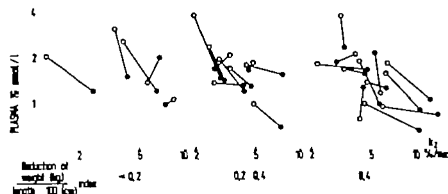


Fig. 2. Relationship between the i.v. fat tolerance and plasma TG concentration in obese subjects before (O) and after (●) operation. The subjects have been divided into three different groups on the basis of the weight loss between the two studies. Logarithmic scales.

seem to occur after operation, suggesting that the subjects move along a hyperbolic correlation line corresponding to a straight line in a double logarithmic system. The general weight reduction pattern of the obese patients in this study has recently been described elsewhere (14). In Fig. 2 the changes have been correlated to weight loss, expressed as reduction of the weight/height index. The observed weight change from one investigation to another depends on the rate with which the patients lost weight and the interval between studies. The Figure shows the relation be k_2 and plasma TG after varying individual loss of weight.

l cases show a marked improvement of their k_2 and a marked plasma TG reduction, although the weight loss may be small. Others have reduced their body weight considerably without any major effect on plasma TG or k_2. In this connection it should be pointed out that the k_2 value is highly reproducible when repeated in the same subject at different intervals (9). This inconstant pattern raises the question whether the effects of the jejuno-ileal shunt operation on plasma TG and k_2 at least in part may depend on other factors than the loss of adipose tissue. One may speculate whether the operation induces changes of the blood flow in the splanchnic region, e.g. to the intestinal blind loop and that removal sites in this area may be perfused more or less depending on the location.

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RED CELL 2,3-DIPHOSPHOGLYCERATE IN OBESE PATIENTS

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Abstract. Erythrocyte 2,3-DPG has been found to be significantly increased in group of 22 obese patients. This is interpreted as the manifestation of compensatory mechanism, secondary to the respiratory distress, promoting oxygen release from the red blood cells through decreased Hb affinity for oxygen.

The association of obesity respiratory insufficiency and polycythemia was originally described nearly 40 years ago (16). Later several investigators have drawn attention to secondary polycythemia in cases of obesity with a certain degree of respiratory insufficiency but without overt pulmonary or cardiac disease (5 18, 26). Hypoventilation with hypercapnia, hypoxemia, and polycythemia associated with obesity has been described as a clinical entity "the Pickwickian syndrome (7). In such patients polycythemia has been interpreted as compensatory to the respiratory insufficiency and has been found to decrease or disappear following weight reduction. It has been suggested that diaphragmatic elevation may cause the hypoventilation in obese subjects. Thus Scherrer and Liedtl (21) have found reduced lung volumes due to a mechanic restriction comparable to what happens during pregnancy or by patients with ascites" They also demonstrated a slight degree of arterial hypoxemia in supine position which disappeared when the patients were standing. There is insufficient knowledge as to the degree of obesity initiating respiratory insufficiency

In the present work erythrocyte 2,3-diphosphoglycerate (2,3-DPG) has been measured in 22 obese patients. A compensatory elevation of 2,3-DPG promoting the oxygen release from the red cells through decreased Hb affinity for oxygen might then be expected, similarly to what happens in hypoxic conditions due to other causes (14 17 25 27).

MATERIAL

22 obese patients, 17 women and 5 men, without signs of pulmonary or cardiac disease were studied. Clinical data are given in Table I. The degree of overweight was estimated according to Lindberg et al. (19). A control group included 24 healthy volunteers, 11 women and 13 men, aged 16 to 45 years (mean 27).

METHODS

Venous blood was drawn from the antecubital fossa into 10 ml Vacutainer tubes containing 143 USP units sodium heparin. Within 2–3 min 1 ml blood was placed in 4 ml chilled perchloric acid, homogenized and extracted. The extracts were neutralized with potassium bicarbonate and assayed for 2,3-DPG by the enzymatic method of Eriksson and de Verdier (11). All analyses are performed in duplicate.

The following additional determinations were made for each subject: ESR, erythrocyte count, Hb concentration, Hct, MCV MCH, MCHC, standard bicarbonate and base excess (BE). Student' *t*-test was used for statistical analysis.

RESULTS

The mean 2,3-DPG value for the normal group was 4.88 ± 0.4 mmol/l erythrocytes. For the obese patients the mean value was 5.27 ± 0.5 mmol/l erythrocytes (Table I). The difference between the two groups is significant ($p < 0.01$).

In 13 patients with an overweight $> 50\%$, 2,3-DPG was 5.39 ± 0.6 mmol/l erythrocytes. The corresponding value for 9 patients with overweight $< 50\%$ was 5.10 ± 0.4 In the second group the increase of 2,3-DPG was not significant ($p > 0.2$).

When the results for the control subjects were analysed for a male and a female group separately no difference was found (Table II). Even when the subjects were grouped into smokers and non-smokers, no significant difference was found in the level of erythrocyte 2,3-DPG

In the obese group five patients had slightly elevated standard bicarbonate (Table I). BE was decreased in one and slightly increased in two. Hb was elevated in three patients (Table I) and Hct in one patient. All other laboratory determinations

Table I. *Clinical and laboratory data of 22 obese patients*

Pat. no	Sex	Age (y.)	Weight (kg)	Height (cm)	Excess weight (%)	2,3-DPG (mmol/l RBC)	Hb (g/100 ml)	Standard bicarbonate (mEq/l)	Other diseases
1	♀	49	119 4	172	> 50	6.32	12.7	26.5	
2	♂	40	112.2	175	> 50	6.14	15.4	25.0	
3	♀	63	93.3	158	> 50	5.91	15 1	26 0	
4	♂	56	146.5	180	> 50	5 79	13.9	27.5	
5	♀	30	102.0	173	45-50	5.63	13.9	22.5	
6	♀	47	126.3	174	> 50	5.60	16.9	25.0	
7	♀	45	92.2	167	45	5.59	14 6	22.5	
8	♀	49	101.5	159	> 50	5.51	14.2	26.5	
9	♀	20	98.7	168	> 50	5.38	12.3	25.0	
10	♀	34	87.8	167	35–40	5.33	14.9	19.5	
11	♀	41	122.5	163	> 50	5.22	12.7	24.0	Diabetes
12	♀	49	93.7	171	40–45	5.19	13.7	25.0	
13	♀	63	105.0	160	> 50	5 13	15.3	24.5	
14	♀	23	88.9	167	35–40	5.07	14.6	23.5	
15	o	19	97.0	178	30–35	5.03	14.2	23.0	
16	♀	63	102.5	161	> 50	4 97	12.2	22.0	Diabetes
17	♀	63	113.7	164	> 50	4.88	14.5	25.0	
18	♂	22	91.0	174	30–35	4.77	16.3	25.0	Diabetes
19	♂	31	107.0	183	40–45	4 77	15.1	24.0	
20	♀	35	97.0	164	> 50	4.57	14 0	22.5	
21	♀	75	119.0	168	> 50	4.57	14.9	27.5	Hypertension
22	♀	67	89.8	164	45	4.50	14 7	27.5	
Mean values		44	105.0			5.27 ± 0.5 (S.D.)			
Normal values						4.88 ± 0.4 (S.D.)	11.6–16.6	19–26	

showed values within normal limits. No correlation was found between erythrocyte 2,3-DPG and the laboratory findings.

DISCUSSION

Erythrocyte 2,3-DPG has been found to be elevated in hypoxic conditions of various causes (17 25 27). *This elevation has been interpreted as the result of* a compensating mechanism that tends to increase oxygen release from the RBC to peripheral tissues through decreased Hb affinity for oxygen. The present results indicate that the relative respiratory insufficiency (6, 7 8, 9 15 16, 18, 21 22, 23) frequently encountered in obesity might trigger the compensatory mechanism mentioned.

Inverse correlation between 2,3-DPG and Hb has been reported (14) not only among anemic patients but also among normal persons. In view of this fact, it may be worthwhile to mention that no difference in Hb level was found between the group of obese patients and the group of healthy volunteers. For both the value was 14.37 g/100 ml. Even by grouping into males and females, no difference was found between the obese and the control group.

Our patients did not represent the most severe degrees of obesity encountered in clinical work. As a matter of fact, several had a relatively moderate degree of obesity (Table I). Nevertheless erythrocyte 2,3-DPG was found to be elevated in the patient group.

Among obese patients there is a certain incidence of *secondary polycythemia (5 18 26), reported* to vary between 0.8 and 5.0% (2) and subsiding when the weight is reduced and hypoxemia disappears (18). However in a series of 50 patients, Alexander et al. (2) did not observe polycythemia. In the present group the Hct was elevated in one of 22 patients. It may therefore be concluded that polycythemia is rather uncommon in obese patients. The determination of erythrocyte 2,3-DPG is obviously a more sensitive test for hypoxemia in obese subjects since 7 of 22 patients showed values above the normal range.

The incidence of erythrocyte 2,3-DPG elevation corresponds fairly well to the frequency of lowered oxygen tension (about 1/3 of the patients) found by Freyschuss (12) in a group of obese patients.

No significant difference in the level of erythrocyte

Table II. *2,3-DPG values from 24 normal subjects, grouped into smoking and non-smoking females and males*

	N	2,3-DPG (mmol/l RBC)
Total	24	4.89±0.4
Females	11	5.00±0.4
Smokers	6	4.85±0.4
Non-smokers	5	5.19±0.4
Males	13	4.78±0.4
Smokers	6	4.82±0.4
Non-smokers	7	4.73±0.4

2,3-DPG was found between smokers and non-smokers (Table II). A disturbance of the compensatory elevation of 2,3-DPG among obese patients, might have been expected since cigarette smoke contains carbon monoxide which has been found to cause a decrease of 2,3-DPG concentration in vitro (3, 13) and in vivo (4). Our results are in accordance with those of Torrance et al. (24) who found no significant difference in the level of erythrocyte 2,3-DPG between smoking and non-smoking normal subjects.

Three patients had diabetes and one hypertension. In all probability neither condition contributed to the 2,3-DPG elevation. Ditzel (10) has found a decreased 2,3-DPG concentration when erythrocytes were incubated in a medium containing 50 mmol glucose and several authors (1, 20) have demonstrated normal erythrocyte 2,3-DPG in diabetics.

As mentioned initially the degree of obesity which may cause respiratory insufficiency is incompletely known. The present results indicate that an increased level of erythrocyte 2,3-DPG may be an early sign of respiratory dysfunction in these cases.

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CELLULARITY AND CELL PROLIFERATION RATES IN HUMAN BONE MARROW

I. An In Vivo M thod to Estimate the Total Marrow Cellularity in Man

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Abstract. The total marrow cellularity using ^{59}Fe as marrow label, has been measured by an in vivo technique including suppression of radioiron reutilization by inactive iron. The blocking efficacy was studied from the red cell incorporation of ^{59}Fe given after marrow sampling. The error of the method was approximately 16%. The total number of nucleated bone marrow cells averaged 21 10^9/kg b.wt. There were 13×10^9 granulocyte precursors, 3.4 10^9 nucleated red cells and 0.016 10^9 megakaryocytes. The marrow reticulocytes averaged 4.6 10^9/kg b.wt., or about 20% of the total marrow cells, which was more than in the circulation (3.1 10^9). The transit time of marrow reticulocytes was calculated to be about 50 hours. About 2 red cells and 400 platelets were produced for every granulocyte and each megakaryocyte produces approximately 300 platelets/day. The total volume for erythro- granulo- and megakaryopoietic marrow cells was approximately 500 ml. The erythroid marrow was calculated to be able to expand maximally by a factor of 10.

The total cellularity of the human bone marrow has previously been estimated with a radioactive iron technique (7 8 11 14 35) or calculated from erythrokinetic data (16, 24 25). Other quantitative methods have also been used, which, how ever are not applicable under clinical conditions. The weight and distribution of marrow in human cadavers have thus been estimated (22, 37), and measurements have been made of marrow olume and cellularity in animal experiments (15 17 38).

Employing the isotope dilution principle with ^{59}Fe as a marrow label, as originally described by Suit (35), an in vivo method for measurements of the marrow cellularity is described in the present report. The technique includes blocking of ^{59}Fe recirculation by carrier-iron injections.

The purpose was to measure the total number of erythroblasts, granulocyte precursors and megakaryocytes and their proliferation rates.

This report describes the method and its use in a group of patients with non-haematological diseases.

In later articles generation and transit times for red cell precursors will be reported both for controls (32) and for patients with primary and secondary polycythaemia (33).

Presented in part to the Congress of the International Society of Hematology 1970.

MATERIAL

Twelve patients with non-haematological diseases were investigated. Since the present studies involve hospitalization, patients giving their informed consent were studied rather than healthy volunteer controls. The patients were generally out-patients with mild cardiovascular disease and with normal haematological findings in the peripheral blood and in the bone marrow. It is seen in Table I, however that two of the patients had moderately decreased Hb values. Since the serum irons, the iron bindings and the stainable iron in bone marrow sections were within the normal limits, it was assumed that iron kinetics were normal. Although there was no evidence of bleeding, infection, haemolysis or impaired renal function, it is recognized that the values presented here are valid for patients with non-haematological diseases and may differ from those found in entirely healthy persons.

METHODS

Bone marrow cellularity

Principle. The bone marrow is labelled with ^{59}Fe. The total marrow cellularity is then estimated from the mean radioactivity per marrow cell and the total radioactivity

Table I. *Clinical data*

Pat. no.	Age (y)	Sex	Diagnosis	Hb (g/100 ml)	Hct (%)	ESR (mm/h)	Serum iron (μg/100 ml)	Iron saturation (%)	Marrow iron[a]	Bone marrow cellularity in section[b]
1	60	♂	Angina pectoris	13.0	40	13	95	33	+	nc
2	56	♂	Diab. mell. levis	14.9	46	9	120	41	++	nc
3	42	♂	Hypertonia ess. ben.	15.1	40	23	162	42	++	nc
4	54	♂	Spondylosis cerv	12.3	38	17	136	47	+	Slightly hrc
5	60	♂	Angina pectoris	13.2	39	14	72	21	+	nc
6	67	♀	Hypertonia ess. ben.	13.2	40	5	108	38	+	Slightly hoc
7	48	♀	Neuropathia	12.0	40	12	100	31	+	nc
8	62	♂	Hypertonia ess. ben.	15.6	46	3	164	55	+	nc
9	56	♂	Bronchitis chron.	15 1	52	4	140	36	+	nc
10	59	♂	Hypertonia ess. ben.	13.3	45	14	79	38	+	Slightly hrc
11	41	♂	Thrombosis cruris	13 4	44	13	71	29	+	nc
12	51	♂	Angina pectoris	14.5	44	2	86	30	++	nc

[a] Stainable reticular iron in bone marrow: + = normal.
[b] nc = normocellular; hrc = hypercellular; hoc = hypocellular

in the whole marrow. The average radioactivity per nucleated bone marrow cell is estimated in marrow sample taken at the time of minimum peripheral blood ity. The total marrow radioactivity at this time assumed to be equal to the total radioiron incorporation into the erythrocytes 12 days later after the blocking f iron recirculation with inactive iron.

Administration of ^{59}*Fe.* Approximately 10 μCi of ^{59}Fe as ferric citrate were injected intravenously in isotonic saline. The specific activity was 8.2 29.8 mCi/mg iron. The exact amount of injected activity was estimated by measuring the radioactivity of the injection set (syringes, needles and swabs) between tw 3 5" crystals before and after the injection of ^{59}Fe.

Handling of marrow sample. At the time of maximum marrow labelling, i.e., about 18–20 hours after the injection of ^{59}Fe, bone marrow aspiration was performed in the sternum or the iliac crest, in some cases in both areas. In order to obtain sample without clumps of marrow cells, the aspirated sample was shaken and filtered under pressure through a nylon mesh with pore size of 80 μ (Swiss Nylon Bolting Cloth, No. 210,01, Kamm, Stockholm). All glass was siliconized and rinsed with 1% MgK_2-EDTA solution; the nylon mesh was likewise rinsed. The sample was centrifuged and, after removal of the supernate, washed twice in 1 1.1% saline. This concentration was found to cause less cell damage than isotonic saline.

Cell counting. Counts were made in the final cell suspension. The total number of nucleated cells and erythrocytes was counted in Buerker counting chamber and, in some cases, also by lectronic counting in Coulter counter. In counting of nucleated cells 0.1% aqueous brilliant cresyl blue was used, as proposed by Harrison (14), to reduce clumping f cells. Smears were stained with May-Grünwald-Giemsa and a differential count of minimum of 1 000 nucleated cells was performed. Cells were classified according to Wintrobe (36). In some cases smears were also made of the total sample, of the nylon mesh and of the filtered sample in order to examine the effect of washing and filtration on the cell distribution. Bone marrow reticulocytes were counted in smears stained with 1% brilliant cresyl blue; 1 000 erythrocytes were counted.

T calculate the total number of megakaryocytes, the ratio of erythroblasts to megakaryocytes was estimated in 4 μ thick sections of bone marrow stained ith haematoxylin-eosin. The average distance between the top of one section and the next section was 20 μ. Since the average megakaryocyte diameter is calculated to be 20.8 μ (11), no correction was considered necessary for multiple counting of particular megakaryocyte in more than one section. The counts were performed with light microscopy (40) and at least 1 000 nucleated red cells were counted.

Blood samples. A venous blood sample taken at the same time as the marrow aspiration, was handled, except for the filtration, like the marrow sample. However the washing solution used was isotonic saline. Erythrocytes and reticulocytes were counted as described before.

A venous blood sample, taken 12 days after injection of ^{59}Fe, was haemolysed and the Hb concentration was determined.

Radioactivity measurements. A 2 / ″ 2′ well type scintillation NaI (Tl) crystal was employed, using integral counting. The radioactivities were measured in the total marrow sample, the marrow supernate, plasma and washing solutions. They were similarly measured in the initial and late blood samples. The radioactivity measurements were corrected to the time for radioiron injection. The samples were counted for 30–100 min. Background and samples were measured alternatively in order to minimize the possible effect of background variation on the error in the bone marrow radioactivity measurements.

T study the efficiency of the marrow blockade with inactive iron comparison was made between the incorporation of ^{59}Fe and that of ^{55}F (100 μCi) given after initiating the marrow blockade. A liquid scintillation counter for simultaneous counting of ^{55}Fe and ^{59}Fe was employed for radioactivity measurements (15).

Calculations. The total marrow cellularity was calculated as follows:

$$TMC = \frac{SC \quad TMA}{SA} \tag{1}$$

here TMC = total marrow cellularity SC = marrow sample cellularity TMA = total marrow radioactivity SA = marrow sample radioactivity

In this simple calculation the SA was corrected for radioactivity attributable to circulating reticulocytes as follows:

$$SA_{corr} = SA - \frac{BA \quad MR}{BR} \tag{2}$$

here SA_{corr} = corrected marrow sample activity BA = peripheral blood sample radioactivity at the time of marrow sampling, MR = number of marrow sample reticulocytes, BR = number of peripheral blood sample reticulocytes.

Corrections were also made for the contribution to the SC from circulating granulocytes. This number, as ell as the number of circulating reticulocytes in the marrow sample was calculated from leucocyte, red cell and reticulocyte counts in the peripheral blood and red cell and reticulocyte counts in the marrow sample.

Blocking of iron recirculation

Any uptake of ^{59}Fe after the time of marrow sampling would lead to an overestimation of the total marrow radioactivity and hence the total marrow cellularity. For this reason iron recirculation was suppressed, as previously (18, 20, 34), by giving inactive iron daily for 12 days starting immediately after marrow sampling. The iron was administered as an intramuscular injection in doses of 100 mg iron in an iron-sorbitol-citric-acid complex (Jectofer, Astra, Sweden) and $FeSO_4$ tablet containing 100 mg Fe^{++} as a daily evening dose. The patients tolerated these iron doses well. Patients with abnormally low unsaturated iron binding capacities were not included in this study

Blood cellularity

The total number of circulating cells was calculated from the cell number per μl and the blood volume. For red cells from the total body Hb, measured by the alveolar carbon monoxide method (31), and the mean corpuscular Hb. An even distribution throughout the blood volume, even for platelets, was thus assumed. The platelet count was performed by phase microscopy

Table II. *Distribution of nucleated cells in 12 bone marrow samples*

Cell type	Medium	% of all cells (mean ± 1 S.D.)	Observed range of all cells (%)
Nucleated red cells	Smear	17.3 ± 3.6	12.0–22.9
Granulocytes[a]	Smear	63 8 ± 7.8	52.1 73.1
Miscellaneous	Smear	18 9 ± 9.3	6.2–33 4
Megakaryocytes[b]	Section	210·1 ± 55	274–87 1

Corrected for circulating mature granulocytes.
[b] Nucleated red cell: megakaryocyte ratio.

Turnover rate of circulating cells

These figures were obtained from the peripheral cell count and the survival time of the circulating cells. The red cell life span as estimated from the endogenous production f carbon monoxide as described earlier (9).

The granulocyte turnover rate was calculated according to Cartwright et al. (4) and Cronkite and Vincent (6) from TBGP λ, where TBGP = total blood granulocyte pool, λ = fractional turnover rate per hour for compartment from which there is random loss.

Cartwright et al. (4) found an average ratio of circulating granulocyte pool to total peripheral (circulating and marginal) granulocyte pool of 0.44 and half-time of 6.7 hours for autotransfused DF^{32} P-labelled granulocytes. Using these figures and the number of cells in the circulating granulocyte pool the granulocyte turnover rate was calculated.

The platelet turnover rate was also calculated from the total number of platelets corrected for recovery or splenic sequestration (12) and assuming an average figure for the life span of 9 days (1).

Volumes of erythro- granulo- and megakaryopoietic marrow cells

The cell volumes used were not measured in this study but taken from assumed (2, 6) or measured (12) average figures given previously for erythroblasts (2) 250 μ^3 for marrow reticulocytes (2) 120 μ^3 for granulopoietic marrow cells (6) 400 μ^3 and for megakaryocytes (12) 4 700 μ^3

RESULTS

Marrow sample cellularity

The number of nucleated cells in the cell suspension ranged from 11 to 76 × 10^6/ml. The distribution of nucleated cells is shown in Table II. The

Table III. *Radioactivity measurements in 12 bone marrow and blood samples 18 h after radioiron injection (mean values)*

	Distribution of radioactivity	
	(cps)	(%)
Bone marrow sample		
Whole sample, activity/ml	2.4	
Activity in supernate	0.4	17.6
Activity in cell suspension	2.0	82.4
Cell suspension, activity/ml	3.3	
Calculated erythroblast activity	2.9	87 1
Calculated marrow reticulocyte activity	0.1	2.0
Calculated circulating reticulocyte activity	0.3	10.9
Blood sample		
Whole sample, activity/ml	0.24	
Activity in plasma	0.13	54.0
Activity in red cells	0.11	46.0

figures are in good agreement with the numbers given earlier (5 16).

For the later calculations it is important that the cell distribution is affected neither by the site of aspiration nor by the sample preparation. Therefore the percentages of erythroblasts and the distribution of erythroblast maturation stages in sternal marrow samples were compared to those in samples from the iliac crest. Only three pa tients would submit to this; in these no significant were noted. Nor was there any significant effect upon the distribution of erythroblasts through the preparation of the cell suspension in smears from the whole sample, the nylon mesh, the filtrate and the cell suspension. The distribution was unchanged except for a slightly higher proportion of proerythroblasts in the cell suspension than in the whole sample (mean value 3.2 and 1 8% respectively).

Marrow and blood radioactivities

A study was made as to whether at the time of marrow aspiration, most of the radioactivity is really in the nucleated bone marrow cells rather than in the supernate, the reticulocytes or the peripheral erythrocytes.

The radioactivity in bone marrow and blood was 10 times higher than in blood alone (Table III). Most of the radioactivity in the final cell suspension was calculated to be derived from nucleated cells, and only 2% from marrow reticulocytes. If it was assumed that all the radioactivity in nucleated cells is derived from red cell precursors, the radioactivity per erythroblast and per marrow reticulocyte would average 0.233×10^{-4} cps and 0.0037×10^{-4} cps, respectively

Very small percentages of the total activities were recovered in washing solutions of marrow samples, and these figures are included in the values for activity in the marrow supernate. The marrow supernate had a radioactivity about 3 times that of plasma activity per ml blood sample.

Table IV *Total numbers, volumes and turnover rates of proliferating and non-proliferating bone marrow cells*

	Total cell number cells/kg b.wt. 10^9 (mean ± 1 S.D.)	Ratio bone marrow circulating cells	Ratio proliferative non-proliferative marrow cells	Turnover rate of circulating cells (h/kg b.wt. 10^7)	Total calculated olume of marrow cells (ml/kg b. t)
Erythropoietic system					
Nucleated red cells	3.4 ± 0.9		1 4		
Marrow reticulocytes	4.6 ± 1 1	3 100			1.5
Circulating reticulocytes	3.1 ± 1 1				
Erythrocytes	264.0 ± 47.3			9 1	
Granulopoietic system					
Granulocytic precursor cells	13.0 ± 5 7	33 1	1 3.2		5.2
Circulating granulocytes	0.4 ± 0.1			9.3	
Megakaryopoietic system					
Megakaryocytes	0.016 ± 0.008	1 1000	1 : 1		0.1
Platelets	15.1 ± 3.8			9 7	

Table V *Errors in a single estimate of the marrow cellularity*[a]

Symbol	Abbreviation for	Order of magnitude	Coefficient of variation[b]
SC	Total no. of nucl. cells/ml	10^{9}	9
THb	Total body Hb (g)	10^{2}	2.6
$BE_{1,2}$	No. of red cells in peripheral blood/ml	10^{9}	3.4
ME	No. of red cells in marrow sample/ml	10^{9}	3.4
MCH	Mean corpuscular Hb (g/μg)	10^{-10}	2.3
BR	Reticulocytes in peripheral blood (%)	10^{-2}	10
MR	Reticulocytes in marrow sample (%)	10^{-2}	10
$BA_{1,2}$	Radioactivity in peripheral blood (cps/ml)	10^{-1}–10^{2}	2.3
SA	Radioactivity in marrow sample (cps/ml)	10^{4}	2.3
	Total coefficient of variation		15.4

[a] The total coefficient of variation is based on the equation estimating th number of nucleated cells:

$$\frac{SC\ THb\ BA_1}{BE_1\ MCH\left(SA - \frac{BA_1\ ME\ MR}{BE_1\ BR}\right)}$$

[b] Calculated from $S.D. = \sqrt{\Sigma d^2/(2n)}$, where d equals the difference of duplicate estimates and n equals the number of duplicate estimates.

Total marrow cellularity

When the average radioactivity per nucleated bone marrow cell and the approximate total marrow radioactivity at the time of marrow sampling were known, the total cellularity could be calculated. From this number and the distribution of cells in the smears and sections of bone marrow the absolute number of the different marrow cells was calculated. The total number of nucleated bone marrow cells was found to average 21.0×10^{9} cells/kg b.wt. (Table VI).

There were 13.3×10^{9} granulocyte precursors, 3.4×10^{9} erythroblasts and 0.016×10^{9} megakaryocytes per kg b.wt. (Table IV). The latter figure was calculated from the ratio erythroblast: megakaryocyte which was found to average 210:1 (Table II).

It is seen in Table IV that the ratio between marrow and circulating cells for erythropoietic cells was 0.03 for granulopoietic cells 33 and for megakaryocytes and circulating platelets 0.001. Thus the percentage of cells localized in the marrow was for the erythron 3 for granulopoietic cells 94 and for megakaryopoietic cells 0.1%. The ratio of proliferative and non-proliferative marrow cells averaged for erythropoiesis 1:4 for granulopoiesis 1:3.2. The figure of 1:1 for megakaryopoiesis is based upon the assumption that 50% of the megakaryocytes are producing platelets (3). The turnover rates of the circulating cells were found to average 9.1, 9.3 and 9.7×10^{7} cells per hour and kg b.wt. for red cells, granulocytes and platelets, respectively.

The total marrow cell volume per kg b.wt. could be calculated from individual cell volumes given and total cell numbers (Table IV).

Error of the method

The ^{59}Fe technique involves several computations and measurements. To study the error of the method duplicate determinations were carried out of the various laboratory steps. The results of these studies are described in Table V which indicates that the error of the method was 15.4%.

DISCUSSION

The total number of marrow cells was calculated from the average radioactivity per nucleated bone marrow cell and the approximate total marrow radioactivity. It is relevant to discuss whether the technical conditions were satisfactory.

Representativity of the marrow sample

A valid expression of the average radioactivity per nucleated cell in calculating the total marrow cellularity requires a marrow sample which is representative of the marrow throughout the body.

Marrow samples from sternum and iliac crest in the same individual did not show any significant differences in these respects. Only three cases were studied, but the results are in agreement with previous findings in man (7) and more extensive studies in animals (13 15 28). Moreover differences in cellularity without changes in the cell distribution would not affect the results.

Accurate cell counting and radioactivity measurements require a homogenous cell suspension, which was obtained by filtration through the nylon mesh. The staining technique described above gave a cell suspension without clumps of cells.

A higher proportion of proerythroblasts was found in the cell suspension than in the whole marrow smear However this would affect the results only slightly since the percentage of proerythroblasts is small, and since they contain comparatively small amounts of Hb. For basophilic erythroblasts and later stages there was an unchanged distribution of cells during the preparation of the marrow sample.

A certain degree of cell damage seems unavoidable during the filtration and washing. This is shown by the finding in the marrow supernate of radioactivity which exceeds what could be calculated to be due to plasma activity alone. Even when, instead of isotonic saline, 1–1 1% saline was used during the preparation, there was still radioactivity in the supernate although the amount was reduced and smaller than reported earlier (7 14). Donohue et al. (7) studied the origin of this activity and concluded that it was mainly derived from broken cells and present as Hb iron. If it is assumed that some cells are completely broken, the supernate radioactivity would not affect the measurement of the radioactivity per marrow cell. However if there is any appreciable breakdown of cytoplasm from cells with intact nuclei, and if these naked nuclei are not retained in the mesh, they would be included in the total nucleated cell count and thus cause an overestimate of the total marrow cellularity For this reason smears from the mesh, filtrated sample and the final cell suspension were examined. A few damaged cells with naked nuclei were seen. It may be assumed that appropriate correction for the radioactivity leakage would result in total cell numbers and volumes about 10% less than those given in Table IV

A dilution of the marrow sample with circulating blood is unavoidable when making bone marrow aspirations. Therefore corrections for the contribution to the cell counts and the amounts of radioactivity in the marrow sample from circulating blood were made. In previous studies, using marrow samples from ribs removed at operation, these contributions were ignored (7 8 14 26). To correct for the circulating reticulocyte activity it was assumed that all the radioactivity in washed peripheral blood cells could be attributed to reticulocytes and that circulating reticulocytes were as radioactive as those in the marrow This assumption was made in spite of previous reports of a 20% higher uptake of ^{59}Fe in marrow reticulocytes than in circulating reticulocytes (35). This would, however not seriously affect the results because the total reticulocyte contribution could be shown to be quite small.

Total marrow radioactivity

The central problem in applying this technique is to estimate the proportion of injected radioiron in the marrow at the time of marrow sampling. In earlier studies a figure was simply assumed for this proportion (11 14, 35), the estimate of which depends mainly on radioiron recirculation.

In the present studies attempts were made to obtain a more precise figure for this proportion. Firstly recirculation of iron was suppressed and, secondly the degree of suppression was measured by comparing the incorporation of ^{59}Fe and that of ^{55}Fe given after initiating the marrow blockade. The ratio of incorporation of ^{55}Fe to that of ^{59}Fe suggests a remaining recirculation and, hence, an overestimation of the total marrow radioactivity and the marrow cellularity by about 10% However only 4 cases were studied and further results with varying doses and times for the administration of inactive iron and the second isotope will be presented separately (31).

The incorporation of ^{59}Fe was on an average 78% of the injected dose. Thus in non-heamatological patients approximately 70% of the injected ^{59}Fe was localized in the marrow at the time of sampling. This figure is in close agreement with an erythroid marrow localization of 66% used in previous studies (7 8 11 14). This figure was arrived at by extrapolating data of carcass lo-

Table VI. *Quantitative studies of human bone marrow*

Author	Method	Marrow cells/kg b.wt. $\times 10^9$: Total nucleated cells	Erythroid marrow	Granulocytic marrow
Osgood (24)	Erythrokinetic	46.0	8.6	25.7[a]
Patt (25)	Mitosis data	13.6	3.4	8.3
Killmann et al. (16)	Mitosis data		5.2 (3.5)[b]	13.1 (8.8)[b]
Suit (35)	^{59}Fe labelling (cell suspension)		4.6	
Donohue et al. (7, 8)	^{59}Fe labelling (cell suspension)	18.0	5.0	11.4
Harrison (14)	^{59}Fe labelling (cell suspension)	10.4–11.1	2.5–3.0	
Harker (11)	^{59}F labelling (tissue section)		2.9	
Present report	^{59}Fe labelling (cell suspension)	21.0	3.4	13.0

[a] Exclusive of mature granulocytes.
[b] Values for different mitotic times, "best estimate" given within parentheses.

calization of radioiron in three animal species to man (7). The total marrow erythron deposit of ^{59}Fe, given 24 hours prior to disarticulation of the lower skeleton, could be calculated to be 84% (23).

The present method does not seem to make it necessary to assume that in controls all the marrow radioiron is in red cell precursors. Radioiron in the reticuloendothelial cells (10) or white cell precursors (29) would still be included in the mean-radioactivity-per-nucleated-cell estimate.

However difficulties arise when the method is used in some pathological conditions. All the radioactivity recovered in erythrocytes 12 days after radioiron administration must have passed the bone marrow. If, however, radioiron is retained in the bone marrow, e.g. in the reticuloendothelial cells in secondary anaemias (10), the total marrow activity will be underestimated and so will the total cellularity. Similarly if there is very considerable haemolysis so that radioactive erythrocytes disappear prior to the 12-day sample, an underestimate will ensue.

Total marrow cellularity

From the total marrow cellularity and the distribution of cells in the sample and section of bone marrow the absolute number of the different marrow cells was calculated. A comparison with results from previous estimates of total nucleated, erythrocytic and granulocytic marrow is shown in Table VI. The present figures are in reasonable agreement with those previously published (7, 8, 11, 14, 16, 24, 25, 35), in spite of the fact that the previous studies were based on different assumptions (16, 24, 25) or different methods of measurement (7, 8, 11, 14, 35) and that no healthy individuals were included in the previous studies with ^{59}Fe.

Number and function of marrow reticulocytes

The total number of marrow reticulocytes averaged 4.6×10^9 cells per kg b.wt., while Donohue et al. (8) reported a corresponding figure of 5.7×10^9. These seem to be the two only quantitative studies of the total marrow reticulocyte number in man.

Marrow reticulocytes are about 20% of the total marrow cells. A corresponding figure of 27% was reported by Seip (30), who suggested that the reticulocytes should be included in calculations of the myeloid-erythroid ratio when one attempts to judge the erythropoietic activity. This would bring this ratio close to 1:1. The present calculations of the ratios between nucleated and non-nucleated red cells and the rate of red cell production make it possible to calculate the marrow transit time of reticulocytes at about 50 hours (37). The number of marrow reticulocytes corresponds to about a 2-day requirement of red cells, which is some-

what more than previous estimates of 1 day in man (35) and 0.75 day in animals (38).

The calculated radioactivity per erythroblast was on an average 63 times higher than the corresponding figure per reticulocyte. The ratio between the numbers of true marrow reticulocytes and nucleated red cells averaged 1.38 : 1. This number is in good agreement with previous figures (26, 30). The ratio together with the average radioactivity per cell indicates than an appreciable portion of heme is synthesized in the reticulocyte stage. This may confirm earlier findings that the high heme content in some reticulocytes (27) is caused by disturbed maturation, possibly by "skipped division" during rapid regeneration.

Number and function of megakaryocytes

The total number of megakaryocytes averaged 0.016×10^9 cells/kg b.wt. Previous estimates of megakaryocyte numbers have varied by a factor of 300 (11).

From the platelet turnover rate (Table IV) and assuming that 50% of the number of megakaryocytes are producing platelets, each megakaryocyte could be calculated to produce approximately 300 platelets per day. It could also be calculated that for each granulocyte . erythrocytes and 400 platelets are produced.

marrow volume

is also possible from marrow volume studies calculate the theoretically possible expansion of a specific cell line, for instance the granulocyte precursors in some leukaemias, and the effect of this on the marrow production rate (6). A simple calculation from the data found in this study indicates a maximum expansion of the erythroid marrow by a factor of 10, assuming that one half of the bone marrow is fatty and that the total volume is 3 000 g. Using data from animal studies a corresponding figure of 6 was calculated for man (38).

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CELLULARITY AND CELL PROLIFERATION RATES IN HUMAN BONE MARROW

II. *Studies on Generation Times and Radiothymidine Uptake of Human Red Cell Precursors*

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Abstract. In group of 12 patients without haematological diseases the total number of erythroblasts has been estimated with ^{59}Fe-dilution technique including the blocking of ^{59}Fe recirculation by carrier-iron injections. The total number of proerythroblasts, basophilic, polychromatic and orthochromatic erythroblasts, was estimated on the basis of the total erythroblast number and marrow differential count and was found to average altogether 3.4×10^9/kg b.wt. The total daily erythrocyte production was estimated by the alveolar carbon monoxide method. Based on the above data the generation times for the various erythroblasts were calculated to be about 11 hours for the most immature and 26 hours for the most mature. The transit time of the orthochromatic erythroblasts was estimated to be 20 hours and that of marrow reticulocytes 51 hours. The total marrow transit time from proerythroblast to circulating reticulocyte was calculated to be 124 hours or about 5 days. Simultaneously in vitro labelling of the erythroblasts with tritium labelled thymidine (H^3TDR) was studied. Of the basophilic erythroblasts 70% were labelled, but only 30–40% of the proerythroblasts and the polychromatic erythroblasts. No simple parallelism is thus found with this technique between the labelling index and the generation time.

To understand the nature of haematological disorders with an increased or decreased production of red cells, it is naturally necessary to know the proliferation rates of the red cell precursors in normal erythropoiesis. These rates have previously been studied by several different methods (2, 3 5 6, 7 11 14 15 16, 19 21 22, 24 25 26, 33).

The methods and results are reviewed in Table I. Calculations of red cell precursor generation times (i.e. the sum of interkinetic and mitotic times) have been based on, among other methods, radiothymidine (H^3TDR) labelling of erythrocytic precursors and on the relation between the total number of erythroblasts and the total erythrocyte production.

In the present study these two methods were used simultaneously in the same individuals. This article describes the methods used and presents and evaluates the findings in a group of patients with non-haematological disorders. Later articles will deal with the results in a group of patients with primary and secondary polycythaemia (31) and in one with different forms of anaemia (30).

MATERIAL AND METHODS

Bone marrow cellularity

Twelve patients with non-haematological diseases were investigated. The material as well as an in vivo technique, using ^{59}Fe as marrow label, for estimating the total cellularity was presented in preceding article (29). The method will be summarized. About 18 hours after i. administration f approximately 10 µCi of ^{59}Fe as ferric citrate a marrow sample was aspirated. In homogeneous cell suspension free from extracellular iron, obtained through filtration and washing, the average radioactivity per nucleated cell was estimated. The total marrow radioactivity at this time was assumed to be equal to the radioactivity of the total erythrocyte mass 12 days later. Because of radioiron recirculation this assumption causes an overestimate of the total marrow radioactivity and hence of the total marrow cellularity. For this reason the iron recirculation was blocked with inactive iron (17 29). Even so, the total cellularity is overestimated by on an average, 10% (17).

Based on these measurements, and after corrections for the contamination of the marrow sample from circulating radioactivity and for cell numbers, the total number of marrow cells was calculated. From this

Table I. *Estimates of red cell precursor generation and transit times*

Author	Method	Generation time (h) Proerythroblasts	Basophilics	Polychromatics	Transit time (h) Orthochromatics
Osgood (21)	Red cell turnover rate (tissue culture)		48[a]		
Weicker (33)	Regeneration rate after aplastic crises or in pernicious anemia	24	24	24	
Patt (22)	Mitosis data		5.75[b]		17.3[b]
Alpen and Cranmore (2)	^{59}Fe grain count half-time	17–20[c]			
Bond et al. (5)	H^3TDR labelling			15–18	19[d]
Fliedner et al. (11)	Mitosis data		22[d]		
Cronkite et al. (7)	Mitosis data		11.2 33.5[e]		
Lajtha and Oliver (16)	^{32}P and ^{14}C-adenine labelling in tissue culture	20	20	30	30
Astaldi (3)	Tissue culture		18–24	12 24	24–36
Rubinstein et al. (26)	H^3TDR labelling	5.2	7.3	23	25
Pollycove and Mortimer (24)	^{59}F labelling			33.6[f]	
Reizenstein (25)	Total red cell production	2.4	11.3	28	
Killman et al (14)	Mitosis data	19.8–39 6[d]	13 6–16.3[d]	10.8–11.5[d]	15.5–16.6[d]
Killman et al. (15)	Mitosis data	49[g]	18.6–95[g] 12.4–63.5[h]	13.1 37.5 18 8–25[h]	
Cronkite (6)	Mitosis data	23[i]	9.5–48.5[i]	6.2 19.2[i]	
Cronkite (6)	H^3TDR labelling	24		9–10[e]	
Cronkite (6)	H^3TDR grain count half-time	24	24	24	
Najean et al. (19)	H^3TDR labelling	19	38	29	22
Present report	Total number of erythroblasts and total red cell production	11	16	26	20

[a] ... for nucleated erythrocyte in the marrow
[b] Proliferating class (5.75); postmitotic stage (17.3).
[c] Observations in the dog.
[d] Turnover time.
[e] Average generation time for all mitotable erythroblasts (Tm 0.5–1.5 h).
[f] Mean erythron hemoglobinization time.
[g] Compartment transit time for mitotic time of 1.14 hours; [h] 0.75 hours; [i] 0.58 hours, from observations in the dog: Oderitchenko et al. (20)

number and differential cell counts in the marrow sample the absolute number of the different red cell precursors was calculated. The calculated error of the method was 15.4%.

Red cell production

The daily erythrocyte destruction and hence, in steady state, red cell production was calculated from the total erythrocyte number and the erythrocyte survival time. The total erythrocyte number was obtained from the total body Hb, measured by the alveolar carbon monoxide method (28), and the mean corpuscular Hb. The erythrocyte life span was calculated from the endogenous production of carbon monoxide (10).

Generation and transit times

Calculations of the generation times were based on the known numbers of cells within the different compartments of the erythroid marrow and on the red cell production per unit time. The formulas used have, in part, been presented earlier (7 25). They were arrived at by making certain assumptions concerning the erythropoietic model.

The erythron consists of proliferating and non-proliferating pool in the marrow and pool of circulating reticulocytes and red cells. The various maturation stages are called proerythroblasts (*P*), basophilic (*B*), medium or polychromatic (*M*) and orthochromatic (*O*) erythroblasts, marrow reticulocytes (*MR*), blood reticulocytes (*BR*) and erythrocytes (*E*). The following assumptions were made:

(*a*) Between the proerythroblast, which is the earliest recognizable precursor cell, and the marrow reticulocyte there are four morphologically distinguishable stages of erythroblasts (*P B M* and *O*).

(*b*) In each stage in the proliferating pool (*P* *B* and *M*) there is one division.

(*c*) All mitoses are heteroplastic; then both daughter cells will be different from the mother cell.

(*d*) Ineffective erythropoiesis and maturation without division are assumed to be negligible in the present patients, although it is recognized that under some pathological conditions they are not.

The flow rate of cells from one phase to another in the proliferating pool, in a steady state, could be expressed as

$$N_{out} = N_{in} + N$$

where N_{out} = flow rate of cells leaving the compartment, N_{in} = flow rate of cells entering the compartment, N = birth rate within the compartment.

N equals $\frac{N}{t}$

where N = number of cells in compartment, t = generation time indicated by subscript.

According to assumptions and *d* the N_{out} for proliferating compartment could also be expressed as

$$\frac{2N}{t}$$

Furthermore, according to assumptions *a* and *b* N_{out} from the last dividing compartment must equal the number of erythrocytes entering the circulation, which in steady state must equal the number of red cells destroyed per day

Thus the flow of cells leaving the polychromatic compartment could be expressed as

$$M_{out} = E_{in} = \frac{2N_M}{t_M} \tag{1}$$

or

$$M_{out} = E_{in} = B_{out} + M = \frac{2N}{t_B} + \frac{N_M}{t_M} \tag{2}$$

The flow rate entering the polychromatic compartment could be expressed as

$$M_{in} = B_{out} = \frac{2N_B}{B} \tag{3}$$

or

$$M_{in} = B_{out} = P_{out} + B_r = \frac{2N_P}{t} + \frac{N_B}{t_B} \quad (4$$

From these equations the formulas for calculation of generation times are obtained:

$$M = \frac{2N_M}{E_{in}} \tag{1}$$

$$t_B = \frac{2N_B \cdot t_M}{N_M} \tag{1, 2}$$

$$\frac{2N}{N_B} \tag{3, 4}$$

The transit times through the marrow non-proliferating compartment (*O* *MR*) were calculated from the number of cells in the compartment under consideration and

Table II *Distribution of human erythroid cells* (*n* = *12*)

	Percentage of all erythroblasts (Mean ± 1 S.D.)	Total cell no. cells/kg b.wt. 10⁹ (Mean ± 1 S.D.)
Proerythroblasts	3.8 ± 0.9	0.12 ± 0 02
Basophilic erythroblasts	11.1 ± 2.8	0.36 ± 0 08
Medium or polychromatic erythroblasts	35.4 ± 6 6	1 16 ± 0.24
Orthochromatic erythroblasts	46.6 ± 9.9	1 72 ± 0 69
Total no. of nucleated red cells		3.4 ± 0.9
Marrow reticulocytes		4.6 ± 1 1
Erythrocytes		264 0 ± 47.3
Erythrocyte life span (days)	*112 ± 9 (1 S.D.)*[a]	
Erythrocyte production rate (cells/h/kg b.wt. 10⁹)		9 1 ± 1 7 (1 S.D.)

Excluding 4 smokers, where life span was assumed (120 days) and not measured.

the red cell production per unit time (E_{in}). Thus the transit time (tr) could be expressed as: for the orthochromatics

$$tr_O = \frac{N_O}{E_{in}} \tag{5}$$

and the marrow reticulocytes

$$tr_{MR} = \frac{N_{MR}}{E_{in}} \tag{6}$$

Radiothymidine labelling of erythrocytic precursors

A bone marrow sample, taken at the same time as described above, was transferred to siliconized tube and coated with 1% MgK_2-EDTA solution. ³HTDR (Schwartz-Bio Research) with specific activity of 3.0/mmole, in maximum amount of 1 μCi/ml of bone marrow sample, was added, followed by incubation for 1 hour at 37°C. The smears were made on gelatin-coated microslides, with red sable hair "0" artist' brush, which was periodically rinsed in polyvinyl-pyrrolidone (PVP Periston-Bayer). Smears are fixed in absolute methanol for 2 hours with one change.

The preparations were then covered with Kodak AR 10 stripping film according to technique modified after Pelc (23) and exposed for 12 days in black boxes in the dark at 4°C. The processing was performed with Kodak K 19B developer and Kodak F 52 acid fixer. The slides were then stained with May-Grünwald-Giemsa. At least 1 000 cells were counted and additional cells belonging to the erythroblastic proliferative pool were counted to obtain at least 200 cells in this pool. Cells with 7 grains or more were counted as labelled. The amount of ⁵⁹Fe given does not cause cell labelling in the autoradiographs.

RESULTS

Table II shows the distribution of cells in the erythron. Approximately 3% of the cells belonging to the erythron are localized in the bone marrow. The relative proportion of cells from proerythroblasts to orthochromatics averaged 1 3 9 16. The ratio between proliferative (proerythroblasts, basophilic and polychromatic erythroblasts) and non-proliferative (orthochromatic erythroblasts and marrow reticulocytes) red precursor cells was approximately 1 4. The bone marrow contained about 1.5 times as many reticulocytes as the entire blood volume and there were on an average 1.4 times as many reticulocytes in the bone marrow as there are erythroblasts. From the total erythrocyte mass and the red cell survival time an average red cell production per hour and kg b.wt. of $9.1 \times 10^7 \pm 1.7$ (1 S.D.) was obtained.

The generation and the transit times as well as the total marrow transit time and the labelling indices are shown in Table III.

The average generation time was for proerythroblasts 11, basophilic erythroblasts 16 and polychromatic erythroblasts 26 hours. The transit time for orthochromatic erythroblasts averaged 20 hours and for marrow reticulocytes 51 hours, [illegible] about 2 days need. The total time from [illegible] to circulating reticulocyte was [illegible] to be 124 hours or approximately 5 days [illegible]ange 81–168 h).

Forty-three per cent of the proerythroblasts were labelled and corresponding figures found for basophilic erythroblasts and polychromatic erythroblasts were 70 and 35% respectively.

Table III. *Generation and transit times and labelling indices (H^3TDR) for human red cell precursors ($n = 12$)*

Mean ± 1 S.D., S.E.M. within parentheses

	Generation and transit times (h)	Labelling indices (H^3TDR) (%)
Proerythroblasts	10.9 ± 2.1 (0.6)	43 ± 7 (2.0)
Basophilic erythroblasts	16.0 ± 3.4 (1.0)	70 ± 12 (3.5)
Medium or polychromatic erythroblasts	26.0 ± 5.7 (1.6)	35 ± 11 (3.2)
Orthochromatic erythroblasts	19.6 ± 8.5 (2.5)	
Marrow reticulocytes	51.2 ± 12.3 (3.6)	
Marrow transit time: proerythroblast to peripheral reticulocyte	123.7 ± 28.0 (8.1)	

DISCUSSION

Since the present studies involve hospitalization, patients giving their informed consent were studied rather than healthy volunteer controls. The investigated patients had, in general, mild cardiovascular disorders and no sign of any haematological disease. The limitations in the patient material have been discussed (29).

The total number of erythroblasts, which averaged 3.4×10^9/kg b.wt. is in good agreement

Table IV. *Labelling indices of human red cell precursors*

Author	*Isotope*	*Administration*	Labelling indices (%) *Proerythroblasts*	*Basophilics*	*Polychromatics*
Bond et al. (5)	H^3TDR	In vivo	83 (B_1) 64 (B_2)[a]	57	42
			100 (B_1) 71 (B_2)[b]	86	40
Bond et al. (4)	H^3TDR	In vitro		50[c]	
Lajtha and Oliver (16)	^{32}P	In vitro	70	50	33
Cronkite (6)	H^3TDR	In vivo	100 ($E_1 + E_2$)[d]	78	33
Cronkite et al. (8)	H^3TDR	In vivo	100 (E_1) 77 (E_2)[d]	82	50
Lundmark (18)	H^3TDR	In vitro	52	63	42
Schmid et al. (27)	H^3TDR	In vitro	67	61	23
Najean et al. (19)	H^3TDR	In vitro	62	63	34
Present report	H^3TDR	In vitro	43	70	35

[a] Two earliest identifiable stages B_1 and B_2 after 30 min; [b] after 60 min.
[c] Younger erythroid cells.
[d] Proerythroblast (E_1) and large basophilic normoblast (E_2).

with previously published figures (9 12, 13 15 21 22, 32). Although the distribution of the erythroblasts is in agreement with the numbers given earlier in the studies of erythrokinetics (8, 15), it is well recognized that the erythroblast percentages in the literature (1) show a considerable variation.

The total red cell masses and red cell survival times were calculated from alveolar carbon monoxide data. During the course of the study it became increasingly difficult to exclude the possibility of CO contamination either because of smoking or of air pollution. Such contamination would not affect the total erythrocyte masses obtained but did preclude the calculation of the red cell life span. Therefore, in smokers, the life span was assumed to be 120 days (Table II).

The generation times, especially for the earlier maturation stages obtained in the present study were shorter than some of those obtained earlier with different methods and assumptions (Table I). For this reason it is of particular interest to examine what changes in the present assumptions would yield longer times.

However changes in the assumptions (for instance more than 4 maturation stages, "skipped division") generally seem to result in even shorter times. If the true erythroblast numbers were less than those estimated, this also would result in even shorter generation and transit times. Moreover even calculations based on "standard man" survival times and cell numbers (25) resulted in considerably shorter generation times than those obtained previously Finally the present generation time relations between the various maturation stages appear a little more logical than some of the earlier data, where appreciably longer generation times were found for the immature than for the mature erythroblasts (Table I). In summary it is suggested that the present generation times, particularly when corrected for the overestimation of the total number of erythropoietic cells, may be more correct than some longer times previously obtained by other methods.

The labelling indices are in reasonable agreement with earlier data (Table IV). The higher labelling of proerythroblasts in vivo than in vitro could be due to either inhibition of labelling by in vitro conditions or to a longer H^3TDR availability in vivo. If the latter explanation is correct, then the lack of parallelism between labelling indices and the calculated generation times is of interest. Previously it has frequently been assumed (5 6, 16, 19 26) that the labelling index simply reflects the generation time. The lack of parallelism could be explained if the DNA synthesis time in the proerythroblasts is shorter than in the more mature cells. In this case the assumption that H^3TDR labelling indices reflect generation times is not permissible.

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CELLULARITY AND CELL PROLIFERATION RATES IN HUMAN BONE MARROW

III. *Studies on Bone Marrow Cellularity and Erythrokinetics in Primary and Secondary Polycythaemia*

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Abstract. Overproduction of red cells in primary and secondary polycythaemia could be due to each erythroblast (eb) dividing more often than normally or to the number of eb being increased but their dividing at normal or perhaps even at slower rate than normally. The generation times of the eb have been calculated from their total number and from the daily erythrocyte production. Simultaneously the in vitro uptake of H^3TDR has been studied. Polycythaemia vera (PCV) patients had significant increase in the number of eb (7.7 10^9/kg b.wt.) in comparison with controls (3.4 10^9/kg) and secondary polyglobulias (4.6 10^9/kg). The generation times of the eb in PCV were significantly longer than in controls and patients with secondary polyglobulia (PS). The high erythrocyte production in PCV thus seems to result from an increased eb population dividing no more often than normally. This could be caused in part by stem cell abnormality which possibly also explains the significant increase of granulocyte precursor cells and megakaryocytes. Labelling indices in immature PCV—and mature PS—erythroblasts differ significantly from normal indices, indicating disturbance of their generation cycle. However, no simple linear relationship could be demonstrated between the calculated generation times and the in vitro labelling indices.

Red cell production may increase appreciably in polycythaemia vera (PCV), but it is not known whether a more rapid cell growth, i.e. more frequent cell divisions, explains the increased production or whether it is explained by an increased erythroblast population. To study the pathogenesis of increased erythrocyte production in these states was one purpose of the present study. The other was to estimate quantitatively the total number of erythroblasts, granulocyte precursors and megakaryocytes in primary polycythaemia and secondary polyglobulia (PS) by the same methods as used previously in control patients (9). The generation times of the different bone marrow cell maturation stages will be calculated and compared to those previously found in controls (10).

MATERIAL AND METHODS

Nine patients with PCV untreated at the time of study and 6 with PS secondary to pulmonary disease with hypoxia were investigated and are presented in Table I.

The methods used have been described in detail in preceding articles (7, 9, 10) and will only be briefly summarized here.

Bone marrow cellularity. The total bone marrow cell count was estimated with a ^{59}Fe dilution technique including blocking of iron recirculation with inactive iron (7, 10). About 10 µCi of ^{59}Fe was administered intravenously to the patient and 18 hours later a bone marrow puncture was performed. The radioactivity per nucleated red cell was determined in the filtered and washed bone marrow sample. The total bone marrow radioactivity at this time was assumed to be equal to the total red cell incorporation of ^{59}Fe 12 days later, after blocking of iron recirculation with inactive iron. Approximately 10% of recirculating radioiron escaped the "blockade" (7). From these data the total number of marrow cells could then be calculated. Furthermore, marrow differential counts made it possible to calculate absolute numbers of the different cell types and maturation stages.

Cell proliferation. The generation times for the dividing erythroblast stages were calculated from their corresponding numbers in the whole bone marrow and from the daily erythrocyte production (10). The generation times calculated in this way were compared to the cell's in vitro uptake of tritium-labelled thymidine (H^3TDR). The transit times for the non-dividing precursor stages (orthochromatic erythroblasts and marrow reticulocytes) were also calculated (10).

Table I *Clinical description of the patients*

Pat. no	Age (y.)	Sex	Hb (g/100 ml)/ RBC (mill/mm³)	Total body Hb found/ expected (g)	Blood volume found/ expected (l)	Serum iron (μg/100 ml)/ iron sat. (%)	Marrow iron[a]	Marrow cellularity in section[b]	Megakaryocytes No.[c]	Megakaryocytes Morphology[d]	Spleno-/ hepatomegaly[e]
PCV patients											
1	22	♂	22.9/7.2	1 653/757	7 1/5.8	72/22	+	hrc	incr	hrt	+/+
2	63	♀	18.5/8.2	878/476	4.7/4 1	44/11	+	hrc	incr	hrt	No/no
3	45	♂	17.9/5.8	859/767	4.8/5.9	96/24	−	hrc	incr	hrt	+/no
4	56	♂	18.9/6.5	1 263/747	6.6/5.7	49/26	−	hrc	incr	hrt	++/+
5	42	♂	19 7/6.7	1 386/880	7.3/6 8	116/29	+	hrc	incr	hrt	+/+
6	61	♂	14 5/5.1	1 023/714	7.3/5.3	96/1.	+	hrc	incr	hrt	+/no
7	62	♀	18.2/6.9	825/466	5 4/4.0	101/29	+	hrc	incr	hrt	No/no
8	58	♂	17.5/5.8	814/614	4 7/4.3	96/30	+	hrc	incr	hrt	No/+
9	74	♀	16.5/6.1	829/433	5.2/3.4	25/−	+	hrc	incr	hrt	No/no
PS patients											
10	61	♂	22.1/8.0	964/951	4.2/7.3	54/14	−	hrc			No/no
11	53	♂	16.9/5 4	919/787	5.8/6.1	128/37	+	nc			+/+
12	55	♂	17 7/6.2	533/542	3.9/4.2	43/9	−	hrc		n	No/no
13	72	♂	16.0/5 5	828/818	5.2/5 7	106/31	+	hrc	n		No/no
14	53	♂	17.3/4.9	765/593	4 5/4 2	146/39	+	nc	n	n	No/no
15	73	♂	18.3/6.8	945/611	5.8/4.7	93/29	−	hrc	incr	n	No/no

[a] Stainable reticular iron in bone marrow + = normal.
[b] hrc = hypercellular nc = normocellular
[c] incr = increased; n = normal.
[d] hrt = hypertrophic; = normal.
[e] + = enlarged on X-ray + + = palpable.
[f] Also suffering from psoriasis.

RESULTS

There were generally no difficulties in distinguishing PCV from PS. About two-thirds of the PCV patients had elevated white cell and platelet counts, and a history of bleeding and/or thrombosis was common. So were spleno- and hepatomegaly It is interesting to see how often the after-bath itch is found. About 75% of the patients with PCV had this symptom, but none of the secondary polyglobulias. This has been related to an increased content of histamine (3). The most significant difference between the PCV and PS patients is found when sections of bone marrow are examined (Table I). The PCV cases have a marked increase of cellularity and a marked and statistically highly significant increase ($p < 0.001$) in the number of megakaryocytes, which are quite large and hypersegmented.

The PS patients had a 3- to 15-year history of pulmonary disease. They all had decreased values for arterial oxygen saturation and arterial oxygen pressure. Where examined, urinary erythropoietin was high in PS and low in PCV Only one PS patient (no 14) had been erroneously diagnosed as a PCV and been given ^{32}P 10 years before this study

Bone marrow cellularity The absolute numbers of the different erythroblast maturation stages and the numbers of non-nucleated cells within the erythron are shown in Table II. The results are compared with those obtained in 12 patients with non-haematological diseases, studied by the same methods as used here. The total number of erythroblasts was markedly elevated in the PCV group (7.7×10^9/kg b.wt.) in comparison with the controls (3.4×10^9/kg $p < 0.001$). It was less elevated in PS (4.6×10^9/kg $0.01 < p < 0.05$) In PCV the numbers both of immature (proerythroblasts, basophilic) erythroblasts and mature (polychromatic and orthochromatic) erythroblasts were increased by a factor of about 2.7–2.2. The corresponding figure in PS was about 1.9–1.3

There was a significant increase ($p < 0.001$) in the total number of granulocyte precursors in the bone marrow in PCV (18.7×10^9/kg b.wt.) as compared both to control patients (13.0×10^9/kg) and PS (11.9×10^9/kg). The same was true for

History of			
Thrombosis	Bleeding	Itching	Previous therapy
No	Yes	No	None
No	No	Yes	None
Yes	Yes	Yes	Blood donor
Yes	Yes	Yes	^{32}P busulfan
No	No	Yes^{f}	None
Yes	Yes	Yes	^{32}P busulfan
Yes	Yes	No	None
No	No	Yes	None
No	No	Yes	None
No	No	No	None
Yes	No	N	None
No	Yes	No	None
No	No	N	None
No	Yes	No	^{32}P
No	Yes	No	None

the total number of megakaryocytes in the marrow (0 04 0 02 and 0.01×10^9/kg b.wt., respectively).

In the peripheral blood there were, of course, significantly more platelets and granulocytes in PCV than in PS or in the control group (Table II).

Cell proliferation It can also be seen in Table II that the red cell production rates for control patients, PCV and PS, averaged 9 1 17.9 and $13\ 8 \times 10^7$ cells/h/kg b.wt. respectively Both PCV and PS differed significantly from control patients ($p < 0.001$ and $0.01 < p < 0.05$ respectively).

Statistically significant differences both in generation times and labelling indices were found between patients with and without PCV The generation and transit times for the red cell precursors are shown in Table III. Proerythroblast generation times were longer in PCV and PS than in the control group, but the difference was significant only in PS ($p < 0.001$). There were significantly longer basophilic erythroblast generation times in PCV than in PS patients ($p < 0\ 01$) and in the control group ($p < 0\ 001$). The generation times of polychromatic erythroblasts were significantly longer in PCV than in PS and the controls ($0\ 01 < p < 0.05$).

Table III shows that no simple linear relationship exists between labelling indices and generation times which would permit conclusions about one to be drawn from the other In PCV it was the immature (proerythroblasts) which had a

Table II. *Distribution of bone marrow and blood cells and erythrocyte production rate in primary polycythaemia secondary polyglobulia and in the control group*

Mean ± 1 S D S.E.M. within parentheses

	Total cell no. (cells/kg b.wt. 10^9) Polycythaemia vera ($n=9$)		Polyglobulia ($n=6$)		Control group ($n=12$)	
Erythropoietic system						
Proerythroblasts	0.29±0.07	(0.02)	0.27±0 05	(0.02)	0.12±0 02	(0 01)
Basophilic erythroblasts	0.99±0.2	(0.07)	0 62±0.19	(0 08)	0.36±0 08	(0.02)
Medium or polychromatic erythroblasts	2.76±0.58	(0.19)	1.69±0.73	(0.30)	1.16±0.24	(0 07)
Orthochromatic erythroblasts	3.68±1 44	(0 48)	2.00±0.57	(0.23)	1.72±0 69	(0.29)
Total no. of nucleated red cells	7 7±1.9	(0.6)	4.6±0 8	(0 3)	3 4±0.9	(0.3)
Marrow reticulocytes	9.1±2.0	(0.7)	6 0±1.6	(0.7)	4 6±1 1	(0.3)
Circulating reticulocytes	9.2±3.8	(1.3)	5.5±3.5	(1.4)	3.1±1 1	(0 3)
Erythrocytes	367.6±102.9	(34.3)	436.6±113.7	(46.4)	264 0±47.3	(13 7)
Erythrocyte production rate (cells/h/kg b.wt. 10^7)	17.9±3 1	(1.1)	13.8±3.4	(1 4)	9 1±1 7	(0.5)
Granulopoietic system						
Granulocytic precursor cells	18.7±6.1	(2.0)	11.9±3 4	(1 4)	13 0±5.7	(1.6)
Circulating granulocytes	1.0±0 4	(0.13)	0.7±0.3	(0.12)	0 4±0.1	(0 03)
Megakaryopoietic system						
Megakaryocytes	0.04±0 02	(0 007)	0 01±0.04	(0.016)	0 02±0.01	(0 003)
Platelets	58.0±43.0	(14.3)	14.3±6 0	(2.4)	15.1±3.8	(1 1)

Table III. *Generation and transit times and labelling indices (H^3TDR) for red cell precursors in primary polycythaemia, secondary polyglobulia and in the control group*

Mean ±1 S D S.E.M. within parentheses

	Generation and transit times (h)			Labelling indices (H^3TDR) (%)		
	Polycythaemia vera (n = 9)	Polyglobulia (n = 6)	Control group (n = 12)	Polycythaemia vera (n = 9)	Polyglobulia (n = 6)	Control group (n = 12)
Proerythroblasts	1_.9±3.1 (1.0)	15.8±2.8 (1.1)	10.9±2.1 (0.6)	52±9 (3.0)	46±6 (2.4)	43±7 (2.0)
Basophilic erythroblasts	22.1±2.7 (0.9)	18.0±2.7 (1.1)	16.0±3.4 (1.0)	54±11 (3.7)	62±8 (3.3)	70±12 (3.5)
Medium or *polychromatic* erythroblasts	31.2±6.0 (2.0)	23.9±4.3 (1.8)	26.0±5.7 (1.6)	31±6 (2.0)	45±7 (2.9)	35±11 (3.2)
Orthochromatic erythroblasts	20.9±8.0 (2.7)	15.3±7.1 (2.9)	19.6±8.5 (2.5)			
Marrow reticulocytes	51.0±7.6 (2.5)	45._±15.2 (6.2)	51.2±12.3 (3.6)			
Marrow transit time: proerythroblast to peripheral reticulocyte	138.1±18.7 (6.2)	119.8±20.7 (8.4)	123.7±27.9 (8.1)			

significantly higher labelling index than in the control group whereas in PS it was the mature polychromatic erythroblasts ($0.01 < p < 0.05$ and $0.01 < p < 0.05$ respectively). The labelling index of basophilic erythroblasts was significantly lower in PCV than in control patients ($p < 0.001$).

DISCUSSION

Clinical. Both in PS and PCV an increased red cell mass and an overproduction of red cells are seen (8). However it is not difficult to distinguish between the two forms clinically The elevated white blood cell and platelet counts in PCV the frequency of splenomegaly thrombosis, bleeding, itching and the absence of a reduced oxygen tension in the blood of the PCV patients help to distinguish them from PS patients. The pronounced reduction of fat in bone marrow sections, the statistically significant increase in megakaryocyte numbers and the characteristic morphology of the megakaryocytes in PCV facilitate the differential diagnosis.

Hb concentration and red blood cell values were as high in PS as in PCV although both the total number of marrow cells and the total number of erythrocytes were higher in PCV than in PS. Peripheral values thus do not reflect bone marrow function correctly

The increased total number of granulocyte precursors and megakaryocytes in PCV does not seem to have been demonstrated previously but agrees with the clinical impression of this "myeloproliferative" neoplastic disease.

Cell proliferation. The mechanism underlying increased production of red cells is not understood in PCV It could be brought about either by a more rapid cell proliferation, as suggested by the term "myeloproliferative" or else by an increased population of erythroblasts which divide at a normal or perhaps even slower than normal rate (2, 12) The present study indicates that there is no rapid proliferation the generation times of the erythroblasts in PCV are longer than in the control patients. This finding not only suggests that the term "myeloproliferative disease is not particularly adequate, but it also constitutes additional evidence that tumour cells probably do not proliferate very quickly It is also stated that leucocyte kinetics in PCV are qualitatively similar to the normal (13).

In PS, on the contrary it is well known that an increased formation of erythropoietin increases the rate of differentiation of stem cells into proerythroblasts. Previous studies (8) also suggest that high erythropoietin levels may shorten the marrow transit time. However the generation time of the proerythroblasts was longer than normal in the present study In the other matura-

tion stages there were no significant differences in generation and transit times. The marrow transit time averaged in PS patients 120 hours, while the corresponding figure for the controls was 124 hours.

The changes found in labelling indices do not seem to indicate whether the generation times are increased or decreased. This finding emphasizes that calculations of generation time from the labelling index (5) are rarely valid. Average generation time may however be calculated from the labelling index if all cells under consideration divide and if the duration of DNA synthesis is known. There is so far no proof of two erythroblast populations in PCV with differences in proliferating rates and morphology. Enzyme studies of erythrocytes in PCV may however be indicative of a younger than normal erythrocyte population in these patients (1).

The present finding of changed labelling indices in some erythroblasts thus suggests a disturbance of their cycle, and therefore does not permit a simple calculation of their generation times.

It is suggested that the increased production of erythrocytes in polycythaemia vera is caused by a pathologically increased erythroblast population. There seems to be only one previous quantitative study of the number of erythroblasts in PCV (4), in which also an increased number was found. However these erythroblasts divide no more often than is normal. It is not yet known how the erythroblast population is increased, but a stem cell disease resulting in rapid, erythropoietin-independent production of erythroblasts would explain the results. This is in agreement with previous hypotheses of an intrinsic defect in the stem cells or of disturbed control of their reproduction (6, 11).

A similar hypothesis could explain the megakaryocyte and granulocyte precursor increase.

A selective effect on the stem cells might therefore provide more adequate therapy than that currently used, which inhibits growth of cells not growing well to start with.

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CELLULARITY AND CELL PROLIFERATION RATES IN HUMAN BONE MARROW

IV Studies on Bone Marrow Cellularity and Erythrokinetics in Hypoproliferative Anaemia

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From the Department of Internal Medicine (Section of Hematology), Karolinska Hospital and King Gustaf V Research Institute, Stockholm, Sweden

Abstract. Whole body erythroblast numbers and erythrocyte production have been estimated in 14 patients with hypoproliferative or aregenerative anaemia, 9 with hypocellular or fibrotic bone marrow sections or pure red cell aplasia and 5 with hypercellular bone marrow sections. Numbers and production were calculated on the basis of average bone marrow cell and erythrocyte incorporation of ^{59}Fe. Erythroblast generation times were calculated, based on numbers, production and marrow differential counts. H^3TDR labelling of erythroblasts was simultaneously studied in vitro. Patients with hypocellular marrows had significantly decreased whole body erythroblast number short generation times and normal labelling indices. It is speculated that the low erythroblast number despite normal or rapid multiplication may be explained by stem cell failure. Patients with hypercellular marrows had almost normal erythroblast numbers, significantly prolonged generation times and significantly decreased labelling indices. It is speculated that accumulation of slowly multiplicating erythroblasts in this form of the disease may be related to its previously demonstrated preleukaemic nature.

The obvious fact that erythrocyte production is a function of both erythroblast numbers and erythroblast generation times has previously been pointed out (7, 8). Data on these variables and erythroblast labelling indices in patients without haematological disorders (8) as well as in patients with primary polycythaemia and secondary polyglobulia (9) have been reported. The purpose of the present investigation was to study these variables in patients with hypoproliferative anaemia and decreased red cell production. The data obtained were compared with control data previously described (8).

MATERIAL AND METHODS

Fourteen patients with hypoproliferative anaemia were studied (Tables I and II). The patients were divided, on the basis of the morphology of the bone marrow sections, into two main groups: those with hypocellular marrow or myelofibrosis, and those with hypercellular marrow. One patient had pure red cell aplasia (PRCA).

The first group (pats. 1–9) included those with presumably reduced whole body erythroblast numbers, namely those with hypoplastic or aplastic marrow (pats. 1–6), those with myelofibrosis (pats. 7 and 8) and the patient with PRCA (pat. 9). The second group included patients with unknown whole body erythroblast numbers (pats. 10–14), although the sections of bone marrow were almost fat-free and the cellularity markedly increased.

The methods used have been reported in detail in previous articles (7, 8) and will be summarized here. The principle for estimating bone marrow cellularity is dilution analysis with ^{59}Fe where the average amount of ^{59}Fe per bone marrow cell is measured in marrow aspirate. In previous work (7), the total bone marrow radioactivity at the time of marrow sampling was assumed to be equal to the total radioactivity incorporated into the erythrocytes 12 days later after blocking iron reutilization with inactive iron. In the present patients this assumption was not justified because in these patients with hypoproliferative and secondary anaemia iron may be incorporated in bone marrow reticuloendothelial cells and remain there without being incorporated into erythrocytes. For this reason the total amount of ^{59}Fe in the whole marrow at the time of marrow sampling was assumed to be 66% of the given dose of ^{59}Fe (1).

Calculations of the red cell precursor generation and transit times were based on the number of cells within the different compartments and the rate of red cell production as described earlier (8). However in previous studies, the erythrocyte production rate was based on an assumed steady state and hence equal to the red cell destruction rate (calculated from the red cell mass and red cell life span). In the present patients this assumption was not justified either. Instead, the erythrocyte production rate was calculated on the basis of the following assumptions:

(*a*) A linear relationship exists, in the production interval covered by hypoproliferative anaemias, between erythrocyte production and erythrocyte radioiron utilization.

(*b*) Normal erythrocyte survival is 120 days and normal erythrocyte incorporation of ^{59}Fe is 80% of the given dose of ^{59}Fe.

(*c*) Breakdown of labelled erythrocytes prior to the uptake measurements of ^{59}Fe (12 days after the tracer injection) is negligible.

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(*a*) A linear relationship exists, in the production interval covered by hypoproliferative anaemias, between erythrocyte production and erythrocyte radioiron utilization.

(*b*) Normal erythrocyte survival is 120 days and normal erythrocyte incorporation of ^{59}Fe is 80% of the given dose of ^{59}Fe.

(*c*) Breakdown of labelled erythrocytes prior to the uptake measurements of ^{59}Fe (12 days after the tracer injection) is negligible.

Table I. *Clinical description of the patients*

Pat. no.	Age (y)	Sex	Hb (g/100 ml)/ RBC (mill./mm³)	Reticulocytes (‰)	Hct (%)	Serum iron (µg/100 ml)/ Iron saturation (%)	Marrow iron[a]	Marrow cellularity in section
Hypoproliferative anaemia with hypocellular marrow or myelofibrosis								
1	43	♂	4.0/1.3	24	14	178/61	++	hoc
2	81	♂	5.1/1.8	33	27	130/52	+	hoc
3	74	♀	8.6/2.6	20	30	99/31	±	hoc
4	35	♀	8.7/2.7	30	32	93/31	+	hoc
5	34	♀	7.3/2.6	10	35	76/25	+	hoc
6	68	♂	10.1/3.4	26	32	99/39	++	hoc
7	67	♀	9.9/2.8	64	29	156/64	+	hoc, fibrosis
8	65	♂	9.7/3.1	24	32	67/34	+	hoc, fibrosis
PRCA								
9	82	♂	3.4/1.2	0	13	260/70	+	nc, very few erythroblasts
Hypoproliferative anaemia with hypercellular marrow								
10	70	♀	6.0/2.0	33	21	60/21	+	hrc
11	69	♂	6.7/1.9	20	21	120/41	+	hrc
12	58	♂	5.8/2.0	35	25	174/46	+	hrc
13	58	♂	9.6/2.9	11	28	177/61	+	hrc
14	68	♂	5.4/1.8	1	21	112/57	++	hrc

[a] Stainable reticular iron in bone marrow: + = normal.
[b] nc = normocellular; hoc = hypocellular; hrc = hypercellular

Table II. *Erythrokinetic description of the patients*

	Total body Hb found/expected (g)	Blood volume found/expected (l)	Total erythrocyte mass (cells/kg b.wt. 10⁹)	Erythrocyte life span (d.)	Erythrocyte production rate[a] (cells/h/kg b.wt. 10⁹)	Erythrocyte destruction rate (cells/h/kg b.wt. 10⁹)
Hypoproliferative anaemia with hypocellular marrow or myelofibrosis						
1	210/815	5.0/6.9	81.1	47	6.6	7.2
2	438/870	6.9/6.7	145.6	57	7.4	10.6
3	413/468	4.8/4.0	220.5	79	7.7	11.6
4	425/573	5.1/4.9	193.7	78[b]	8.0	10.4
5	425/473	4.4/4.4	199.1	61	7.7	13.6
6	345/709	3.0/5.1	146.4	n.i.	6.7	n.i.
7	396/541	4.9/5.1	207.9	47	4.7	18.4
8	423/442	4.2/3.8	239.7	80[b]	6.6	12.5
PRCA						
9	274/1013	n.i.	78.4	45	0.008	7.3
Hypoproliferative anaemia with hypercellular marrow						
10	265/432	3.9/4.0	153.0	60[b]	6.9	10.6
11	307/737	5.9/6.2	150.1	n.i.	6.4	n.i.
12	406/807	5.9/6.1	148.1	n.i.	7.0	n.i.
13	421/601	4.8/4.6	237.4	50	7.8	19.8
14	361/843	6.0/6.3	134.0	48	3.9	11.6
Mean ± 1 S.D. for 12 controls				112±9	9.1±1.7	9.1±1.7

[a] Calculated from $\frac{\text{normal RBC mass (/kg b.wt.)}}{\text{normal RBC survival (h)}} \cdot \frac{\text{actual RBC utilization of } ^{59}\text{F}}{\text{normal RBC utilization of } ^{59}\text{F}}$

[b] Estimated with DF^{32}P because in these patients who were smokers carbon monoxide method to estimate RBC life span is unreliable. In all other patients (except nos. 6, 11 and 12, who were smokers) the carbon monoxide method was used.
n.i. = not investigated.

Table III. *Distribution of erythroblasts and erythrocytes and red cell production rate in hypoproliferative anaemias and in the control group (mean ± 1 S.D S.E.M within parentheses)*

Marrow cellularity	Total cell no. (cells/kg b.wt. 10⁹) Hypocellular myelofibrosis (= 8)	Hypercellular (5)	Control group (= 12)
Proerythroblasts	0.03 ± 0 02 (0 01)	0.11 ± 0 03 (0.01)	0.12 ± 0 02 (0.01)
Basophilic erythroblasts	0 17 ± 0.11 (0 04)	0 44 ± 0.17 (0 08)	0.36 ± 0 08 (0.02)
Medium or polychromatic erythroblasts	0.70 ± 0.27 (0.10)	2.14 ± 1 10 (0.40)	1 16 ± 0.24 (0.07)
Orthochromatic erythroblasts	0.31 ± 0.22 (0.08)	0.77 ± 0.34 (0.14)	1 72 ± 0 69 (0.20)
Total no. of nucleated red cells	1.2 ± 0 61 (0.21)	3.3 ± 1 7 (0.76)	3 4 ± 0 88 (0.25)
Erythrocytes	179.3 ± 51.6 (18.2)	164.5 ± 41 4 (18.5)	264 0 ± 47.3 (13.6)
Erythrocyte production rate (cells/h/kg b.wt. 10⁷)	6.9 ± 1 0 (0.4)	6 4 ± 1.5 (0.7)	9 1 ± 1 7 (0 8)

Thus, under these assumptions, erythrocyte production rate, EP (cells/h/kg b.wt.), was calculated as follows.

$EP_f = EP \quad U_f/U$

here f found, = normal and U = erythrocyte utilization of ⁵⁹Fe (%).

In vitro labelling with tritium-labelled thymidine (H^3TDR) of erythroblasts in the marrow sample was performed as described earlier (8), and the labelling indices were estimated.

RESULTS

Whole body erythroblast numbers. The erythroblast percentage (expressed as mean ± 1 S.D and S E.M.) was significantly reduced both in patients with hypocellular marrows and myelofibrosis (9 4 ± 3.4, 1.2) and in those with hypercellularity (11 7 ± 4.8, 2.1) as compared to that of the controls (17.3 ± 3.6, 1.0) ($p < 0.001$ and $0.01 < p < 0.05$, respectively).

As seen in Table III, patients with myelofibrosis and hypocellular marrows had, as expected, a reduced total number of erythroblasts ($p < 0.001$). This seemed to be lower the more pronounced the hypocellularity was. In one case of PRCA the erythroblast number was reduced by a factor of about 350, as compared to the average number for the control patients. In contrast patients with hypercellular marrows did not show any significant difference from the erythroblast numbers in control patients.

In the cases with myelofibrosis and hypocellular marrows, whole body numbers of all the erythroblast maturation stages were significantly reduced ($p < 0.001$). In contrast patients with hypercellular marrows did not show any significant difference in the number of proerythroblasts and basophilics, while they had a significantly higher number of polychromatics ($0.001 < p < 0.01$) and a reduced number of orthochromatics ($0.01 < p < 0.05$).

The average relative proportion of cells for the four erythroblast maturation stages was found to be 1 6 37 12 in the patients with hypocellular marrows and myelofibrosis and 1 4 22 8 in the patients with hypercellular bone marrows. The average ratio in control patients was 1 3 9 16. Thus there is a shift to the left with relatively more immature and fewer mature cells, most pronounced in the cases with myelofibrosis but also in the cases with hypocellular and hypercellular bone marrows.

Erythrocyte production. The average red cell production rate in hypoproliferative anaemia with hypocellular marrows was 6.9 $10^7 \pm 1$ 0/h/kg b.wt. The corresponding average figure for the patients with hypercellular marrows was 6.4 10 ± 1.5. Obviously both figures are significantly lower ($0.001 < p < 0.01$) than those of the controls (9.1 ± 1 7). In the only PRCA case studied the erythrocyte production rate was calculated to be 0.01 10 /h/kg b.wt.

The erythrocyte destruction rate was calculated from actual erythrocyte mass and erythrocyte life span, when available. The red cell life span varied between 45 and 80 days. Because of increased destruction and reduced production an average deficit of 6 10^7 erythrocytes/h/kg b.wt. occurs daily at the present anaemic Hb level. If a normal Hb level were to be maintained with the present red cell life spans, the deficit would be approximately 11 10^7 erythrocytes/h/kg b.wt. Only when the Hb concentration is decreased to about 5–6 g/100 ml can production keep up with destruction. The anaemia is thus caused partly by a decreased pro-

Table IV Generation and transit times and labelling indices for red cell precursors in hypoproliferative anaemia and in the control group (mean ±1 S.D S E.M within parentheses)

Marrow cellularity	Generation and transit times (h)			Labelling indices (H^3TDR) (%)		
	Hypocell., myelofibr (n=8)	Hyper cellular (n=5)	Control group (n=12)	Hypocell., myelofibr (n=8)	Hyper cellular (n=5)	Control group (n=12)
Proerythroblasts	3.4±1.8 (0.6)	11.6±5.0 (2.2)	10.9±2.1 (0.6)	36±16 (5.7)	0	43±7 (2.0)
Basophilic erythroblasts	9.3±5.1 (1.8)	28.6±8.5 (3.8)	16.0±3.4 (1.0)	57±18 (6.4)	20±11 (4.9)	70±12 (3.5)
Medium or polychromatic erythroblasts	20.0±6.2 (2.2)	69.8±32.6 (14.6)	26.0±5.7 (1.6)	27±9 (3.2)	8±5 (2.2)	35±11 (3.2)
Orthochromatic erythroblasts	4.4±2.6 (0.5)	33.0±8.3 (3.7)	19.6±8.5 (2.5)			

duction and partly by an increased destruction of red cells.

Erythroblast generation and transit times. The calculated generation and transit times for the erythroblasts as well as the labelling indices are shown in Table IV In the hypocellular and myelofibrotic group the generation times for the proliferating cells and the transit times for the non-proliferating cells (orthochromatics) were significantly shorter than the corresponding times in the controls. This was more pronounced in the proerythroblasts ($p < 0.001$), the basophilics ($0.001 < p <$) and the orthochromatics ($p < 0.001$) and less d in the polychromatics ($0.01 < p < 0.05$). contrast highly significantly increased generation times ($p < 0.001$) were found for basophilics and polychromatics in the cases with hypercellular marrows, while the generation time for proerythroblasts and orthochromatics did not differ significantly from those in the control group

Labelling indices A similar pattern was found regarding labelling indices, which in the cases with hypocellular marrows and myelofibrosis did not differ from the labelling indices in the control group although they were significantly higher than in the patients with hypercellular marrows. This was most pronounced in the proerythroblasts ($p < 0.001$). In the hypercellular group on the other hand, there were markedly reduced labelling indices ($p < 0.001$).

DISCUSSION

It has previously been stated that it is difficult to measure the whole body number of bone marrow cells in man, even when the iron metabolism is not greatly disturbed (1 2, 3, 4 7 10). When the radioactive iron technique is used, the central problem is to estimate the proportion of the injected radioiron dose localized in the whole marrow at the time of marrow sampling.

In a preceding article (7) the total number of marrow cells was calculated from the average radioactivity per nucleated marrow cell and the total marrow radioactivity at the time of marrow sampling. The latter figure was obtained from the radioiron incorporation value 12 days later after blocking iron reutilization by inactive iron. It was calculated that this technique overestimated marrow cellularity by about 10% (5 7). In this study the total marrow radioactivity was assumed to be 66% of the given dose of ^{59}Fe, according to Donohue et al. (1). This is only an assumption.

Similarly the calculation of erythrocyte production is based on an assumption. There is probably an approximately but not a strictly linear relationship between erythrocyte production and radioiron incorporation. For these reasons, the present estimates of whole body cellularity probably give a larger error than the 16% previously calculated for the controls (7).

All times given in the present report are average times. Further study is required of the possibility that two cell populations with different generation times occur in one patient. The present average times would, of course, be correct for neither population.

In hypoplastic anaemia and myelofibrosis the reduction in the whole body erythroblast number is more pronounced than the reduction in the total erythrocyte production. Thus the calculated average

erythroblast generation times become short. The erythroblasts in this group had normal labelling indices.

In cases with hypercellular marrows the whole body erythroblast number is almost normal, but the erythrocyte production is as low as in the patients with hypocellular marrows. Thus, the calculated average generation times become long. In these cases the labelling indices are decreased.

Almost normal generation times were found earlier in polycythaemia vera (9) and it was suggested that the increased whole body erythroblast number in this disease may be caused by abnormal stem cell proliferation. Similarly it could be argued that in hypoplastic anaemia the reduced whole body erythroblast number in the absence of prolonged generation times, may be attributed to abnormal stem cell proliferation. On the other hand, the prolongation of the generation times and the reduced labelling indices in patients with hypercellular marrows reflect changes in the erythroblast proliferation rates. These are of particular interest since two of the patients later developed acute leukaemia. This form of disease has previously been suggested to be of a preleukaemic nature (6).

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QUANTITATION OF ERYTHROPOIESIS BY A NEW METHOD

III. *The Blocking Effect of Inactive Iron on Radioiron Reutilization*

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Abstract. In new methods for the quantitative estimation of erythropoiesis and bone marrow cellularity using radioiron, inactive iron is given to block reutilization of radioiron. The efficacy of this blocking technique has been studied by giving two radioiron isotopes (^{59}Fe and ^{55}Fe), one before, the other after different doses and forms of inactive iron. It was found that 60% of the radioiron is definitely destined for erythropoiesis, the remaining 40 may disturb quantitation of erythropoiesis and marrow cellularity by reutilization. About half of this iron is reutilized in spite of blocking. Serum iron and reutilization of radioiron are significantly and inversely related. It is calculated that erythropoiesis is overestimated by 10 .. if this blocking technique is used.

Under certain circumstances it is desirable to quantitate erythropoiesis. This can be done either by measuring erythrocyte life span or by estimating plasma iron turnover and red cell utilization of radioiron (4). The first method is often inconvenient because it is time-consuming and requires a steady state during a long period. The second method is much faster but, because of recirculation and re utilization of radioiron, the values will only be semi-quantitative indices of the erythropoietic activity

In animal studies it could be shown that large doses of inactive iron could dilute recirculating radioiron so much that reutilization became insignificant (5 17). As a consequence of this observa tion a method was developed for the quantitative determination of human erythropoiesis, in which the disturbing effect of radioiron reutilization was inhibited by injections of inactive iron (7). By applying this technique to healthy individuals, values were found which overestimated erythropoiesis by only about 10% as compared with normal values taken from the literature (7), whereas the older methods resulted in an overestimation of about 50% (4). In cancer patients such indirect comparisons were performed in a similar way and with similar results (8). The same technique was used in studies of cellularity and cell proliferation rates of human bone marrow (13 14, 15).

The purpose of this study was to find out the degree to which the amounts of inactive iron administered inhibited recirculation and reutilization of radioiron, whether inhibition could be improved, and to study the changes in iron kinetics induced.

Two iron isotopes (^{59}Fe and ^{55}Fe) were used, one was given before, representing the radioiron in an iron turnover study (7), the other after different blocking measures, the second isotope thus representing recirculating iron. The incorporation of both isotopes into the circulating erythrocytes was measured.

MATERIAL

Thirteen patients with different diseases and pparently normal erythropoiesis were investigated (Table I). Except for one case with idiopathic thrombocytopenia, haematological routine values were normal.

METHODS

On separate occasions 5 μCi ^{59}Fe-citrate (specific activity 15–30 μCi/μg Fe) and 100 μCi ^{55}Fe-citrate (specific activity 6–9 μCi/μg Fe) were given. A 20 ml syringe contained the radioiron dose in volume of about 1 ml. After venepuncture, blood was slowly aspirated until the syringe was filled and, to secure complete binding to transferrin, the mixture was slowly and completely injected during about 5 min. This procedure was repeated twice to ensure complete administration of the isotope. An aliquot of each isotope solution was used as standard. Inactive iron in doses of 100 mg was given either as an i injection of an iron-dextran complex (Ferrigen Astra, Sweden), or as an m. injection of an iron-sorbitol-citric-acid complex (Jectofer® Astra, Sweden). In some experiments FeSO tablets containing 100 mg Fe^{++}

Table I. *Incorporation of ^{59}Fe and ^{55}Fe into erythrocytes of 13 patients given various amounts and forms of inactive iron, starting 8 hours after the first isotope*

Pat. no.	Diagnosis	Radioiron incorporation[a] (%)	Injected amount of inactive iron (mg): Before 2nd isotope	Injected amount of inactive iron (mg): Total	2nd isotope	Interval between 1st and 2nd isotope (h)	Serum Fe/TIBC (μg/100 ml)[c]
Intravenous							
1	Obesity	75/75	200	400	[illegible]Fe	32	—
2	Cerebral haemorrhage	60/60	200	400	[illegible]Fe	32	—
3	Obesity	85/20	100	400	[illegible]Fe	17	—
4	Cerebral haemorrhage	70/70	100	400	[illegible]Fe	17	—
5	St. post infarct. myocard.	90/65	100	400	[illegible]Fe	18	50/252
6	Idiopathic thrombocytopenia (normal bleeding time)	70/25	200	1 600	[illegible]Fe	32	116/390
7	Cerebral haemorrhage	84/84	300	1 400	[illegible]Fe	80	52/272
Mean		75/53					
Intramuscular							
8	Gastritis	100/20	100	400	[illegible]Fe	32	88/—
9	St. post cystopyelitis	80/60	200	500	[illegible]Fe	104	72/342
10	Hypertension (benign)	95/65	100	1 300[b]	[illegible]Fe	16	56/330
11	Hypertension (benign)	100/80	100	1 400[b]	[illegible]Fe	32	57/357
12	Hypertension (benign)	70/40	100	1 900[b]	[illegible]Fe	32	100/318
13	St. post infarct. myocard.	89/95	300	1 500[b]	[illegible]Fe	44	42/336
Mean		89/60					
Mean (all subjects)		83/58					

[a] Into circulating erythrocytes. 1st/2nd isotope.
[b] In addition one FeSO tablet containing 100 mg Fe^{++} in the evening.
[c] Before start of the experiment.

were added as an evening dose. The first dose of inactive iron was given 8 hours after the injection of the first isotope. 'hen total of 400 mg inactive iron was given, further …tions were administered 1 4 and 7 days after the first iron dose. When higher total doses of inactive iron given, daily injections were performed until total dose 1300–1900 mg was reached. The interval between the first and the second isotope was varied as shown in Table I.

After the isotope administration, samples of venous blood were taken about three times a week. Radioactivity measurements were made by simultaneous liquid scintillation counting technique described previously (6). The percentage of the injected radioiron incorporated into the circulating Hb was calculated from the average amount found in the blood samples between the 10th and the 17th day of study the total amount of Hb as determined by the alveolar carbon monoxide method (12) and the total amount of radioactivity injected. Serum iron concentration and total iron binding capacity were determined according to Zak and Epstein (18). The results of these studies are presented in T ble I. In 23 other cases with different diseases and without signs of iron deficiency anaemia, in which erythropoesis was quantitated according to the blocking method (7), serum iron was deter mined both t the start and between the 10th and the 17th

Table II. *Serum iron before and after injections of inactive iron, ^{59}Fe incorporation into erythrocytes and plasma iron turnover in patients without signs of iron deficiency anaemia (mean and range)*

	n	Serum iron (μg/100 ml): Before inactive iron	Serum iron (μg/100 ml): After inactive iron	p	Plasma iron turnover (mg/day)	^{59}Fe incorporation (%)
Whole material	23	85 (23–239)	141 (54–257)	0.01–0.001	39.9 (0–98.7)	72 (7 143)
Serum iron below 50 μg/100 ml	7	33 (23–42)	147 (85–250)	< 0.001	20.6 (0–34.6)	96 (72 143)
Serum iron above 50 μg/100 ml	16	108 (52–239)	138 (54–257)	0.2–0.1	48.4 (0–98.7)	62 (7 95)

Mean iron dose 1200 mg.

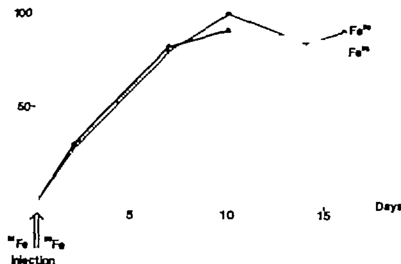


Fig. 1. Percentage radioiron (^{59}Fe – ^{55}Fe) appearing in circulating blood after simultaneous injection of both isotopes.

day of the study (Table II). When the iron determinations were performed during iron administration, the samples were taken immediately before the next iron injection, that is, not earlier than 24 hours after the preceding injection. Iron determination involved no difficulty under these circumstances.

The statistical analyses were performed according to Snedecor and Cochran (16).

RESULTS

Control experiments

To determine the overall accuracy of the technique used, ^{59}Fe and ^{55}Fe were injected in immediate succession into one subject and no inactive iron was given. No significant difference in the utilization of the two isotopes for erythropoiesis could be observed (Fig. 1).

Blocking experiments

The results obtained in 13 blocking studies are shown in Table I. The ratio of the radioiron incorporation of the two isotopes, one given before, the other after inactive iron, showed a considerable variation between individual cases: mostly less of the second isotope was incorporated. The mean incorporation value for all cases taken together was 83% for the first and 58% for the second isotope, the difference being statistically significant ($0.001 < p < 0.01$). Radioiron incorporation did not differ significantly when i.v. blocking was compared with i.m. blocking (1st isotope $0.05 < p < 0.2$, 2nd isotope $p > 0.05$). Neither the inactive iron dose given before the second isotope nor the total iron dose bears any relationship to the proportion of the second radioiron dose incorporated into erythrocytes ($p > 0.2$ and $p > 0.05$ respectively). The total iron dose does not correlate with the incorporation of the first isotope either ($p > 0.80$).

The incorporation of the second isotope was significantly correlated to the serum iron concentration measured before the start of the experiments ($K = -0.891$, $0.001 < p < 0.01$). There was no significant relationship in this material between serum iron and incorporation of the first isotope ($K = 0.608$, $0.05 < p < 0.2$).

In 23 cases with different diseases studied with this blocking technique and lacking signs of iron deficiency anaemia, serum iron values were determined before and at the end of the blocking study. All patients except two received the iron as i.m. injection and the mean dose given was 1200 mg. The mean values are compiled in Table II. It can be seen that, after giving inactive iron, a highly significant increase in serum iron occurs. This change is mainly brought about by a heavy increase in patients having low serum iron values (< 50 μg%) at the start.

In contrast to the smaller material presented in Table I the serum iron values measured at the start of the study correlated significantly with radioiron incorporation ($K = -0.766 \quad p<0.001$). There was no significant correlation between iron values measured at the end of the study and iron incorporation ($K = -0.426 \quad 0.05>p>0.02$).

DISCUSSION

The greatest part of the radioiron leaving plasma is destined for erythropoiesis and within minutes incorporated into Hb (10). Eight hours after radioiron injection the reflux of radioiron into plasma is observed (11). This amounts initially to about 8% and increases to a total of about 34% in the later phase of a 2 weeks observation period in normals (2).

To block this reflux, inactive iron was given starting 8 hours after isotope administration. The aim was to dilute the amount of recirculating radioiron and hence to make reutilization of radioiron insignificant.

The main purpose of this study was to measure the efficiency of this blocking by estimating the fraction of the radioiron which is incorporated into the erythrocytes in spite of these efforts. No turnover measurements were performed in this study as incorporation measurements were considered sufficient to answer our questions, and as the in-
iron was suspected to make meaningful turn-
measurements of the second isotope impossible.
ne first isotope in the present study (I_1) should
ave as the radioiron does in our kinetic methods (7 14). The fraction of this isotope (I_1) which is incorporated into Hb comes mainly from iron directly destined for erythropoiesis (x) and, in addition, a certain unknown amount of recirculating iron escaping the blockade. The mean incorporation value for I was 83%. The incorporation of the second isotope (I_2), which was injected after blocking had started and should behave as recirculating iron, was 58% in the present material. By inserting these values in the equation

$$I = x + I \quad (100 - x)$$

where I = percentage of 1st isotope incorporated into erythrocytes, I = fraction of 2nd isotope incorporated into erythrocytes, x = net percentage of radioiron used for erythropoiesis, x can be calculated.

Accordingly the net amount of radioiron used for erythropoiesis (x) is 60% and consequently the total amount recirculating 40%. This figure is in close agreement with an erythroid marrow localization of 66%, which was arrived at by extrapolating data of carcass localization of radioiron in 3 animal species to man (3).

A reflux figure of 40% is somewhat greater than that reported by others—34% in normals (2). The difference is, however small and may be caused by a tendency to iron deficiency in our material, as in this state reflux was shown to increase (2). As 58% of the recirculating iron (I_2) of a total of 40% escapes blockade, the overall amount of radioiron reutilized is calculated to be 23%. This would mean that, with the type of blocking used, erythropoiesis will be overestimated by 23%. There is, however ineffective erythropoiesis random destruction of erythrocytes, and possibly also destruction of short-living erythrocytes, amounting to about 15% of the total Hb produced in the normal (1 9), which counterbalances this error The resulting overestimation of erythropoiesis should thus, on the basis of the material studied, not be greater than 10%, an accuracy well acceptable for a biological method. This figure is identical with that arrived at earlier (7) and with the results obtained by other more complicated methods (2).

In the larger material (Table II), serum iron values measured at the commencement of the experiment correlate negatively with the incorporation percentage of the first isotope. This finding is explained by the fact that, when trace amounts of radioiron are given, specific activity of serum iron will be high when serum iron values are low and vice versa. When equal amounts of iron are to be used for erythropoiesis, in cases with low serum iron and consequently high specific activity more radioiron will be incorporated into the erythrocytes than in cases with high serum iron, which explains the correlation described above. The fact that the second isotope also correlates negatively with the serum iron concentration measured at the beginning of the study indicates that the differences in specific activity prevailing at the start of the study do not disappear in spite of iron loading. As serum iron increases after administration of inactive iron and as the difference in iron values between those with low and those with high initial values disappears after blocking, as well as the correlation with radioiron incorporation, the specific activity of the recirculating iron must be quite similar in all cases. Thus, the second isotope,

being the indicator of recirculating iron, indicates with its higher incorporation in cases with low serum iron that, not only more radioactivity but more iron also is incorporated in these cases. Thus, blocking efficiency and therefore accuracy of the iron method should decrease in cases with low serum iron.

The fact that the form and dose of inactive iron used for blocking did not influence incorporation of either isotope indicates that blocking was optimum in the dose range studied (400–1900 mg). It must be borne in mind, however, that the doses used in the group to which iron was given intravenously were lower than those given intramuscularly. Thus, whatever blocking technique is used, the present conclusions are only applicable when iron doses are not lower than those used by us.

Studies are in progress to determine the validity of the method in the individual case by means of another independent method, using DF³²P for estimation of red cell survival.

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ERYTHROPOIESIS IN PATIENTS WITH BONE MARROW METASTASES

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Abstract. The erythropoietic part of the bone marrow has been morphologically analysed in 16 anaemic patients with bone marrow metastases and in 16 healthy controls. The proportion of erythroblasts within the blood-forming tissue was identical in the two groups. In the patients with bone marrow metastases the percentage of basophilic erythroblasts was abnormally high and elevated mitotic indices of the erythropoietic precursor cells were recorded. A threefold increase of erythroblastic islands, i.e. erythroblasts in contact with reticulum cells, was found in the patient group. The results indicate that anaemia was not due to a reduced amount of erythropoietic tissue caused by the tumour infiltration of the bone marrow. Nor were there any signs of an impaired proliferative activity of the erythroblasts. It is suggested that "erythroblastic islands" are signs of intramedullary phagocytosis of erythroblasts by reticulum cells and that the impressive occurrence of such formations in the patient group may indicate an ineffective erythropoiesis caused by the malignant disease.

Invasion of the bone marrow by tumour cells has been found to be common in patients with metastatic carcinoma (5). Although widespread cancer is almost invariably associated with anaemia the effect of the tumour cell infiltration on the normal bone marrow cells is incompletely known.

The aim of the present work was to investigate whether any characteristic changes in the composition and mitotic activity of the erythropoietic pool will occur when the bone marrow is infiltrated by metastatic tumour cells. Since in a previous communication (3) it was suggested that erythroblastic islands, i.e. erythroblasts in contact with reticulum cells, are abnormally common in patients with anaemia associated with malignancy or inflammatory lesions, the number of such formations was also estimated in the present series of unequivocally malignant conditions.

MATERIAL AND METHODS

Patients

On routine examination of bone marrow smears obtained by sternal puncture, tumour cells were found in 16 patients, 9 men and 7 women. The age of the patients was 36–84 years (mean 60). In order to decide whether the foreign cells were of tumour origin the cytological criteria given by Söderström (12) were followed. The percentage of tumour cells in the aspirate was less than 5% in 5 patients, 5–20% in 7 and more than 20% in 4. The primary tumour was mammary carcinoma in 4 patients, prostatic carcinoma in 3, bronchial carcinoma in 3, cancer of the gall bladder in 1 patient, pleural mesothelioma in 1 and malignant melanoma in 1. In 3 patients the primary tumour was unknown. Three patients had undergone radiotherapy 1, 19 and 24 months before the sternal puncture and two had been transfused 1 and 5 weeks before the bone marrow examination. No patient was treated with cytostatic agents prior to the investigation.

Controls

Sixteen apparently healthy persons, 7 men and 9 women, with normal haematological data and normal ESR served as controls. Their age was 19–82 years (mean 53). There was no significant difference in age between the patients and the controls.

Bone marrow examination

The bone marrow smears were stained with May-Grünwald-Giemsa. Through examination of 3 000 normal nucleated bone marrow cells the proportion of erythroblasts was determined. 1 000 erythroblasts were then examined and classified according to *Heilmeyer* and *Begemann* (8). Proerythroblasts and basophilic erythroblasts were pooled into one group and denominated basophilic erythroblasts. A mitotic index of the erythroblasts was determined through examination of 1 000 erythroid precursor cells. When erythroblasts were found in close contact with or apparently lying within a reticulum cell the formation was classified as an erythroblastic island and the number of such formations per 1 000 erythroblasts was registered.

RESULTS

The Hb concentrations were significantly lower in the patients compared to the controls (Fig. 1).

The bone marrow smears from the patients were generally rich in cells and in this respect comparable to the aspirates from the controls. On an average 21% of the normal bone marrow cells in the patient group were erythroblasts. In 11 patients the erythro-

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PACEMAKER–VENTRICULAR BLOCKS

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Abstract. Three patients are presented with pacemaker ventricular (P V) or ventricular–pacemaker (V–P) block. All patients had ischemic heart disease and severe heart failure and were treated with transvenous endocardial pacing because of high degree AV block and periods of asystole. One patient had 1st degree P V block for several days before the endocardial electrode was replaced and the P V block disappeared. Another patient as in the terminal phase of an acute myocardial infarction when she developed 1st degree V–P block. The retrograde latency in the conventional leads corresponded to the time difference between bipolar intraventricular and bipolar precordial lead. The third patient was in the terminal phase of myocardial failure when he developed 1st degree P V block followed by 2nd degree P V block type I and type II and finally high degree P V block. The mechanism of P V blocks is discussed.

Constantly or intermittently non-responding ventricles is a not uncommon complication in pacemaker *treatment, and it may be explained by* different causes such as malfunction of the generator dislocation of an endocardial electrode or rise of the stimulation threshold because of the tissue reaction around the electrode. Pacemaker latency which we prefer to call 1st degree pacemaker-ventricular (P–V) block, is a much more interesting arrhythmia, as is the Wenckebach type of incomplete pacemaker capture, which may correspondingly be termed 2nd degree P–V block, type I. The latency may also be retrograde, giving rise to 1st degree ventricular–pacemaker (V–P) block. These arrhythmias seem to be rare, and we have found only one report in the literature on pacemaker latency and incomplete pacemaker capture (1) and none on retrograde latency We shall here present one case each of these three pacemaker arrhythmias and dicuss some pathophysiological explanations.

CASE REPORTS

Case 1

Male, aged 81 During hospitalization because of severe heart failure he developed complete heart block with syncope. A QRS-inhibited pacemaker (Elema 145) with an endocardial electrode as implanted and postoperatively the threshold for stimulation successively increased to 4–6 V within 14 days. At this time monitoring revealed latency of 0.10 sec between the pacemaker stimulus and the ventricular response for 7 days. The pacemaker was set at 10 V and the stimulation rate was increased and decreased, but the latency persisted. Fig. 1 show the effects of decreasing the stimulation rate. The ventricular response slow down with constant latency for three QRS complexes, 1st degree P V block. The next tw R waves do not inhibit the stimulus, which may occur when the stimulation rate is nearly the same as the spontaneous QRS rate. It could alternatively be explained by 1st degree V–P block, which was not, however studied as in case 2.

The heart failure was considered to be of ischemic origin. The patient was treated with 0 13 mg digoxin and 1.2 g quinidine daily The indication for quinidine was some short runs of ventricular tachycardia following the pacemaker electrode application. The plasma concentrations of digoxin *and quinidine ere not determined at the time of the P V* block. The Q–T interval was normal and so were the serum electrolytes. A chest X-ray did not reveal any dislocation of the transvenous electrode in the apical part of the right ventricle. Following its repositioning the stimulation threshold was constantly below 2 V and the 1st degree P V block as not again observed although the patient as still on digitalis and quinidine treatment.

This case demonstrated 1st degree P V block. A 1st degree V–P block was proved in another patient

Case 2

Female, aged 80. The patient was admitted because of an acute anterior myocardial infarction and complete heart block. Asystole supervened immediately after admission, and temporary pacemaker of the QRS-inhibited type (Elema 145) was implanted. Runs of ventricular tachycardia were treated by an infusion of lignocaine 2 mg/min. Seven hours later she died from severe myocardial failure.

Terminally the spontaneous QRS complexes inhibited the pacemaker only if their R waves appeared more than 0.05 sec before the next stimulus (Fig. 2a), but the latency between the stimulus and the induced QRS complex was not extraordinary (0 03 sec). At this time the stimulation threshold was 1 V and the bipolar intraventricular QRS amplitude as far above the sensing threshold. The pacemaker was set t 10 V and the stimulation rate was varied, but the findings



Fig. 1 (5 small squares = 0.1 sec; ink jet recorder; bipolar chest lead.) During successive decrease of the stimulation rate the first 3 QRS complexes follow 0.12 sec after the pacemaker spike which wanders 0.05 sec into the QRS during the next 2 spontaneous complexes before it disappears. 1st degree P–V block.

were the same. To study them further a bipolar intraventricular ECG was recorded from the pacemaker electrode simultaneously with a bipolar precordial ECG over the right ventricle (Fig. 2*b*). The *stimulus was detected at the same* moment in both leads, in the intraventricular lead as a deflection out from the paper. The stimulus activated the ventricles with a latency of 0.03 sec. Regardless of their configuration the spontaneous QRS *complexes appeared about* 0.03 sec earlier in the precordial than in the intraventricular lead, corresponding to a 1st degree V–P block.

The patient had been on 0.25 mg digoxin every second *day with the last dose two days before admission*, and was treated during the pacing with lignocaine. The plasma concentrations were not determined. The Q–T interval was normal, as were the serum electrolytes.

This case suggests that 1st degree V–P block may be one cause among others of malinhibiting pacemakers. The last case demonstrates a short run of 2nd degree P–V block of the Wenckebach type.

Case 3

Male, aged 72. The patient was admitted because of an acute myocardial infarction located anteriorly. The ECG on admission showed right bundle branch block + left anterior hemiblock + 1st degree A–V block, and within the first hour the patient developed several attacks of asystole. A temporary pacemaker was immediately implanted and later replaced by a permanent one; both were of the QRS-inhibited type (Elema 145 and 158). During the third week the patient successively developed severe heart failure followed by hyponatremia and anemia.

Terminally a latency of about 0.13 sec appeared between the stimulus and the ventricular response. 15 min prior to

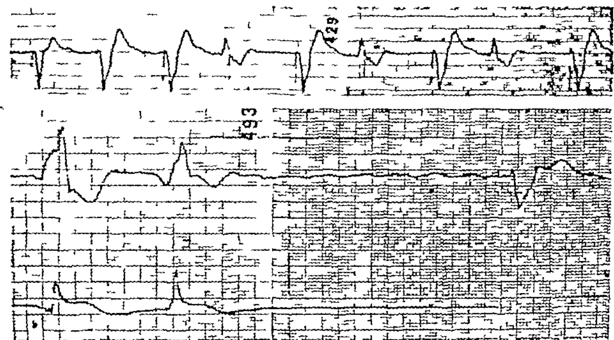


Fig. 2a. (5 small squares = 0.1 sec; ink jet recorder; lead II.) The 6 pacemaker-induced QRS complexes follow 0.03 sec after the "spike". The first 2 spontaneous QRS carry a "spike" 0.05 sec after the onset of the R wave, but after an increase of the pacemaker cycle by 0.02 sec the 3rd spontaneous QRS does not.

Fig. 2b. (10 small squares = 0.1 sec and 1 mV in the upper lead, 5 mV in the lower one; ink jet recorder; bipolar precordial lead above and bipolar intraventricular lead below.) The last QRS complex is preceded by a pacemaker stimulus, simultaneously inscribed as a spike in the external lead and a discontinuation of the internal one. The initial deflection of the 2 spontaneous QRS complexes is 0.03 sec earlier in external lead. (There was no time difference between the two ECG channels.) 1st degree V–P block.

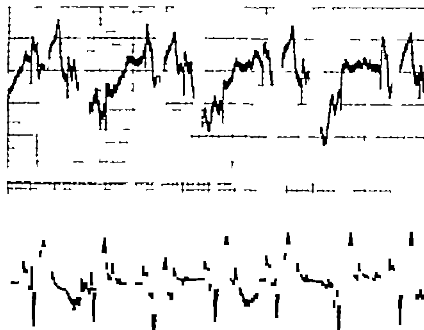


Fig. 3a. (1 small square = 0.1 sec; ink jet recorder; bipolar chest lead.) The first pacemaker "spike" of each Wenckebach cycle is followed by a QRS complex with a latency of about 0.13 sec. The latency following the second "spike" is about 0.17 sec. The third "spike" is completely blocked. 2nd degree P–V block, type I.

Fig. 3b. (1 small square = 0.1 sec; ink jet recorder; bipolar chest lead.) Every second pacemaker "spike" is followed by a QRS complex with a latency of about 0.15 sec. Every second "spike" is completely blocked. 2nd degree P–V block, type I or II.

the final asystole the patient passed a few cycles of P–V block of Wenckebach type with 3:2 conduction (Fig. 3a). This short Wenckebach period was preceded and followed by 2nd degree P–V block of type II with 3:2 or 2:1 conduction (Fig. 3b) and subsequently followed by high degree P–V block. The stimulation threshold at this time was 1.8 V and rise to 10 V did not have any effect on the latency nor on the asystole. The patient was on 0.36 mg ouabain daily but no other cardiotropic drugs. The Q–T interval was normal. The autopsy showed the pacemaker electrode to be properly positioned in the apical part of the right ventricle. The infarct included the anterior and lateral wall of the left ventricle as well as the ventricular septum and the anterior wall of the right ventricle.

DISCUSSION

The pacemaker spike usually merges with the initial part of the induced QRS complex, and it is uncommon to find it precede the initial deflection by 0.05 sec or more. The myocardial response to the pacemaker impulse is all or none once the stimulation threshold is exceeded, and increasing the amplitude of the pacemaker impulse does not change the interval between the pacemaker spike and the QRS complex.

No or intermittent response to the pacemaker stimuli is a common complication in pacemaker treatment. The reason for this is most often an elevation of the stimulation threshold due to dislocation of pacemaker electrode or to tissue reaction around the tip of the subendocardial electrode. A 2nd degree P–V block of type II could be explained by movements of the catheter tip, but 2nd degree P–V blocks of Wenckebach type II are difficult to explain by electromechanical factors, as are P–V blocks of 1st degree. Their explanation must reasonably be bioelectrical disturbances. A possible location of the block is between myocardial and Purkinje cells.

The cause of the block may be advanced myocardial depression by disease or drugs. All the present patients had ischemic heart disease and severe heart failure, in cases 2 and 3 associated with large acute infarcts. Moss and Goldstein (1) reported on 5 patients with different types of P–V blocks and all had significant intrinsic myocardial disease.

All the present patients were on digitalis, case 1 also on quinidine and case 2 on lignocaine. Three of five patients reported by Moss and Goldstein were receiving antiarrhythmic agents.

BOOK REVIEWS

L'Actualité Rhumatologique 1972 présentée au Praticien. Edited by S. de Sèze, A. Ryckewaert, M. F. Kahn and A. P. Peltier. 354 pp. 93 F. L'Expansion, Paris, 1973.

This is the ninth annual volume of review articles (56 authors) from the Centre de Rhumatologie Viggo Petersen, Hôpital Lariboisière, and is intended for practising physicians. The book covers an impressive variety of topics, from Still's disease to chondrocalcinosis, diabetic neuropathy and Paget's disease. Separate chapters also deal with prostaglandins, calcitonin and vitamin D.

For the Scandinavian reader who ventures to cross the language barrier the sections on Whipple's disease, the Tableiro-Weissenbach syndrome, Gold nephrotoxicity and Experimental arthritis will offer interesting reading. The last 40 pages deal with therapeutical problems concerning metastatic bone cancer and this subject is rather completely covered.

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Immunology, Immunopathology and Immunity. By Stewart Sell. 277 pp. $12.95. Harper and Row. Hagerstown, Maryland, USA, 1972.

"The author's aim is to give an "organized, concise yet meaningful presentation of immunology, immunopathology and immunity stressing their interrelationships". This is no easy task within the limited space allowed. The book is intended both for students and for younger and older doctors as an introductory text and may be of value for the last two categories.

The first part, Immunology, deals with the anatomy and physiology of humoral and cell-bound immunity. This section is generally clear and adequate in length. In the description of Ig structure one could have mentioned the J-piece, and some information on alpha- and mu-chain disease would have added to the value of this chapter. The limitations of the classical Bence Jones test should have been stressed. When describing antigen–antibody reactions the Oudin-tube method is described at length (without mentioning Oudin), but nothing is said of the method of radial diffusion (Mancini) which has almost completely replaced it in clinical routine analyses.

The next section, Immunopathology, deals with harmful immune reactions. It starts with a chapter on classification, which is probably hard to digest for an unprepared reader at this stage. It then describes the various humoral and cell-bound types of reactions. In the chapter dealing with cytotoxicity and cytolysis the complement system is presented. One would have expected this in the first section, and it would have deserved more space. The alternate pathway for activation apparently was not delineated when this book was written, showing how fast immunological texts grow obsolete. On the whole the author attempts to cover too much ground in this section, the consequences are often superficial catalogue type of disease presentation, adding littl to the fundamental understanding of the subject. There also seems to be no need t go into details of asthma treatment, including recommendation of psychotherapy. One chapter deals with collagen diseases, among which surprisingly rheumatic fever is included. Muscle wasting is said to occur in only 20% of rheumatoid arthritis patients and RNA antibodies to be common in SLE.

The last section, Immunity, contains well written chapters on immune deficiency, cancer immunity and the Shwartzman reaction. Some minor objections concern Au antigen, which is of course found in only one form of hepatitis, amyloid is present in fewer than 20% of myeloma patients; transition from benign monoclonal gammopathy to myelomatosis may certainly be observed, but is a rather rare event.

The book is illustrated with a number of good schematic drawings and didactic tables. One is disturbed by the large number of misprints, especially in the middle section.

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SERUM PRE-β-1 LIPOPROTEIN FRACTION IN CORONARY ATHEROSCLEROSIS

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Abstract. Using two separate electrophoretic techniques the presence of pre-β-1 lipoprotein fraction in serum has been studied in a series of 46 patients. Twenty-five of these had normal coronary arteries and 21 coronary atherosclerosis documented by angiography. Six patients with normal coronaries (24%) and 11 with coronary artery disease (52%) had pre-β-1 in the serum ($p<0.05$). The distribution of elevated serum cholesterol and/or triglycerides was roughly equal in the two angiographically different groups. The occurrence or absence of the pre-β-1 lipoprotein fraction was related to the family history for coronary heart disease yielding suggestive evidence of a genetically determined lipid abnormality.

In the electrophoresis of lipoproteins separate bands have been observed in the pre-β region (1, 5, 11, 20, 22, 23). With a few exceptions (14, 15, 19) no clinical significance has been attached to this finding. Studies in Boden have revealed, however, that an extra pre-β lipoprotein fraction, called pre-β-1, is prevalent in patients with angina pectoris (9) and after myocardial infarction (8). Family studies have shown that this fraction is genetically determined (10). Since abnormalities in lipid metabolism as risk factors in coronary artery disease (CAD) are related to the coronary atherosclerosis per se and not directly to its clinical manifestations like angina pectoris and myocardial infarction, this study was undertaken to explore the possible relationship of this extra lipoprotein fraction to angiographically documented CAD.

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PATIENTS AND METHODS

Angiographic records of patients studied within the last 6 months in the Helsinki University Central Hospital were reviewed and 60 patients were selected on the basis of reasonable travel distance from the hospital. Forty-six of them could be traced and were willing to participate in the trial. Selective coronary angiography by the Judkins technique had been done in part of the series as a preoperative assessment for subsequent direct myocardial revascularization, and in part of the series for the evaluation of chest pain. For descriptive purposes the obstructions in each main epicardial coronary artery were graded as follows: 0=no obstruction, 1=<25%, 2=25–50%, 3=50–75%, 4=subtotal, and 5=total obstruction. Twenty-five patients had completely normal coronaries with a total score of 0. Twenty-one had obstructions ranging from 2 to 14 with a mean total score of 8. Twenty patients have since been operated upon by the venous bypass technique.

The resting ECG was analyzed by conventional criteria for changes indicating coronary heart disease (CHD). The chest pain and the functional significance of the coronary obstructions were evaluated by a multistage maximal exercise test using an electrically braked bicycle ergometer and constant ECG monitoring. A horizontal or downward sloping ST-segment depression of 1 mm or more was accepted as positive. The chest pain was tabulated as angina pectoris only if typical and confirmed by positive resting and/or exercise ECG. No patient had significant renal, hepatic or thyroid disease. Some characteristics of the series are shown in Table I.

The presence of the following factors was determined: smoking, hypertension (≥160/100 mmHg), diabetes, intermittent claudication, and family history of CHD. The genetic field was restricted to the first degree relatives since information past two generations was incomplete.

Following an overnight fast the blood samples were taken at 8–10 a.m. After centrifugation the serum samples were stored at +4°C and transferred by air to Boden, where they were analyzed on the same day with-

Table I *Some characteristics of the series*
NCA=normal coronaries, CAD=coronary artery disease

	NCA	*CAD*
No of pats.	25	1
Sex (♂/♀)	11/14	17/4
Age (y)*	40 4 3–57	43 7 23–54
*Height (cm)**	*166* 151–184	*168* 143–182
Weight (kg)*	66.8 40–86	66 46–85
Angina pectoris	13/25 (52%)	21/21 (100%)
Previous myocardial infarction	3/25 (12%)	14/21 (67%)
Abnormal resting ECG	4/25 (16%)	18/21 (86%)
Abnormal ercise ECG	11/21 (52%)	16/16 (100%)

Mean and range

out knowledge of the angiographic data. Cholesterol (17) and triglyceride (3) determinations were performed with a Technicon Autoanalyzer Electrophoresis and scanning were done with Beckman microzone electrophoresis system as previously described (7) Cellulose acetate (Gelman Sepraphore III) was used as supporting medium and the lipoprotein fractions were stained according to Kohn (16). Electrophoresis was also per m 0.5% agarose (Behringwerke) and stained with Black B (6). If pre-β-1 lipoprotein fraction was esent it was found by both electrophoretic methods.

Cholesterol exceeding 280 mg/100 ml and triglycerides ≥2.0 mmol/l were accepted as denoting hyperlipidemia. Four patients in both groups were under long-term treatment for their hyperlipidemia. If normolipidemic at the time of study the pretreatment values were used in tabulation. According to personal experience the occurrence of pre-β-1 fraction is not influenced by current lipid-lowering drugs.

RESULTS

Only three patients had claudication and this factor was excluded from the further analysis The prevalence of relevant variables in the two angiographically different groups is shown by Table II The sole significant difference was a more frequent positive family history in patients with CAD The presence of hyperlipidemia was roughly equal

Six patients with normal coronaries (4%) had a positive pre β-1 band in the electrophoresis, in contrast to 11 with CAD (52% $\chi^2=3.971$ $p<0.0$ (Fig. 1) The positive pre-β-1 fraction was the onl lipid abnormality in four patients with CAD (19% and in two patients with normal coronaries (8%).

When the presence or absence of pre-β-1 w related to the family history it became evide that 10 patients in the group with normal cor naries had a negative family history and 9 of (90%) also had a negative finding for pre-β-1 Fourteen patients in the whole series who had a positive pre-β-1 knew their family history In 13 of these (93%) it was positive for CHD (Table III) Pre β-1 positivity was also related to smoking and hyperlipidemia (Table III)

DISCUSSION

Using the coronary angiographic data as the primary criterion in material selection a rather high percentage of patients with unequivocal angina pectoris and some with earlier myocardial infarc tion with normal coronary arteries was found Only obstructions in the main epicardial vessels were used in describing the series but analysis of smaller branches was in accordance with the grouping of patients into two different categories (Table I) A lengthy discussion on the lack of cor

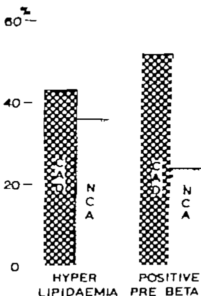


Fig 1 Distribution of hyperlipidemia and pre-β-1 in the groups studied. *CAD*=coronary artery disease *NCA*=normal coronary arteries.

Table II. *Distribution of relevant variables among angiographic groups*

NCA = normal coronaries CAD = coronary artery disease

	NCA	CAD	χ^2	p
Positive family history	14/"4 (63 %)	17/19 (89 %)	5 100	<0.05
Smoking	8/25 (3 %)	11/21 (52 %)	1 943	ns
Hyperlipidemia	9/25 (36 %)	9/21 (43 %)	0.221	ns
Diabetes	5/25 (20 %)	1/21 (5 %)	2.342	
Hypertension	2/25 (8 %)	3/21 (14 %)	0.489	ns

relation between clinically evident CHD and angiographic findings lies outside the scope of this presentation but the great proportion of females in this group is worth emphasizing (Table I). Studies done in some of these patients on regional perfusion with [133]xenon and γ-camera have revealed perfusion deficits in spite of normal coronaries by angiography.

In an earlier study on a population of 286 men subjects with angina pectoris according to a questionnaire or clinical examination had more often a positive pre-β-1 fraction (45 %) than subjects without angina (14 %) (9). The present series consisted of 34 patients with angina pectoris. Thirteen of them had a positive pre-β-1 fraction (38 %). However only 21 patients with angina (62 %) had angiographically visualized CAD and the prevalence of pre-β-1 positivity in these was 52 %. This illustrates the familiar fact that studies relating different variables to CAD on the basis of the clinical manifestations carry an error due to unassessed coronary atherosclerosis.

A number of studies have been performed to elucidate the relation between lipid abnormalities and coronary atherosclerosis documented by angiography (2 4 1 13 18 21). In patients with CAD the prevalence of hyperlipidemia has been in the order of 80 % in contrast to the 10–40 % in patients with normal coronary arteries. It is worth pointing out that gross atherosclerotic changes can be found with entirely normal lipid values and vice versa. Assessment of the pre-β-1 lipoprotein fraction was not included in those studies.

Two reports have recently called attention to the correlation between the pre-β-1 lipoprotein fraction and coronary atherosclerosis (14 19) revealing an extremely high prevalence of positive pre-β-1 in patients with CAD. The data are not, however amenable to detailed analysis of other concurrent lipid abnormalities and factors known to be associated with CAD.

The present findings reveal that while the "customary" lipid abnormalities were almost equally distributed in the two angiographically different groups the pre-β-1 positivity was accumulated in the group with CAD (Fig. 1 Table III) supporting the assumption that this lipid fraction has significance in CAD (8 9 14 19). The earlier genetic study consisting of analysis of two families (10) suggested that pre-β-1 may be determined by an autosomal dominant inheritance. Further family studies in progress have strengthened this thesis. In this context the findings of Knoblock and Hall (15) are of special interest. When studying medical students they found that subjects with dual pre-β peaks in electrophoresis all confirmed a family history of heart disease while subjects with single peaks all had a negative family history. The present data, which show a significant difference in the prevalence of a positive family history with relation to the pre-β-1 lipoprotein fraction are further suggestive evidence of a genetically determined lipid abnormality. Much larger series however are needed to test the hypothesis that CAD in patients with positive

T ble III *Different variables in relation to the presence of pre-β-1 in serum*

	Pre-β-1 positive	Pre-β-1 negative	χ^2	p
Coronary atherosclerosis	11/17 (65 %)	10/"9 (34 %)	3 943	<0 05
Positive family history	13/14 (93 %)	18/29 (62 %)	4.202	<0 05
Smoking	1_/17 (71 %)	6/29 (21 %)	11_11	<0.001
Hyperlipidemia	11/17 (65 %)	7/29 (24 %)	7 410	<0.01
Diabetes	4/17 (°4°)	2/"9 (7 %)	2.639	
Hypertension	_/17 (12 %)	4/"9 (14 %)	0.034	ns

family history but negative customary lipid and lipoprotein data is related to the presence of pre β-1 lipoprotein fraction in the serum The same applies to the clarification of the significance of the relationship of pre β-1 and smoking (Table III)

ACKNOWLEDGEMENT

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PRE β-1 LIPOPROTEIN AND EARLY DETECTION OF RISK FACTORS FOR CORONARY HEART DISEASE

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Abstract. Seventy-five apparently healthy 16–17 year old schoolboys have been studied for the presence of pre-β-1 lipoprotein by two separate methods of lipoprotein electrophoresis. Forty-three per cent of the boys revealed detectable pre-β-1 lipoprotein. The pre-β-1 positive group had higher mean value of total ($p<0.01$) and free ($p<0.01$) cholesterol than the pre-β-1 negative group. A higher mean value of total CO_2 ($p<0.02$) and lower chloride value ($p<0.05$) were also found in the pre-β-1 positive group. Six young males with the most prominent pre-β-1 lipoprotein fraction also showed higher value of hematocrit ($p<0.05$). These results may provide further explanation for the higher incidence of CHD previously found among pre-β-1 positive middle-aged males.

Epidemiological investigations indicate that hyperlipoproteinemia occurs in altogether 15–20% of a male population (16). The types II A, II B and IV according to the typing system of Fredrickson and Lees (14) are the most frequently found. In samples of middle-aged males who have had myocardial infarction these hyperlipoproteinemias may occur in 50–60% of individuals (8, 16). Gross atherosclerotic changes documented by angiography may however be found in subjects with entirely normal lipid and lipoprotein values and vice versa (3).

Using a slightly modified method for electrophoresis of lipoproteins on cellulose acetate membranes an extra slow-moving pre-β" lipoprotein designated as the pre-β-1 fraction, was inconstantly found in clinical routine examinations. This lipoprotein had not previously been used in the classification of lipoprotein abnormalities. Using different electrophoretic methods often after preceding fractionation with ultracentrifugation several investigators however reported high density lipoprotein with pre-β mobility or dual peaks in the pre-β area (13, 23, 28). A pre-β-1 fraction was found in subjects with normal as well as high lipid levels.

Further studies showed that the occurrence of pre-β-1 lipoprotein followed an autosomal dominant mode of inheritance (11). Indications of equilibrium between the pre-β and the pre-β-1 lipoprotein fractions with a possible exchange of lipids and protein were also found (5, 9).

In an epidemiological investigation among 41–60-year-old males, started in 1970, a pre-β-1 lipoprotein fraction was found in 23% of 1,29 investigated subjects. Among the first examined 286 men between the ages of 56 and 60 years a significant correlation between cases with typical or suspect angina pectoris and the occurrence of a pre-β-1 fraction was found ($p<0.001$) (10). A correlation between an extra pre-β lipoprotein fraction and angina pectoris had not been reported earlier.

A recent study of patients with coronary atherosclerosis documented by angiography furthermore gave suggestive evidence of genetically determined lipoprotein abnormality with significance in coronary artery disease (CAD) (15). Papadopoulos et al. (22) utilizing 0.5% agarose found double pre-β bands in 15% of normal asymptomatic patients and in 80% of patients with myocardial infarction. Recently other investigators reported correlation of dual pre-β lipoprotein peaks with history of heart disease (20) and a significant correlation between a pre-β-1 lipoprotein subfraction and moderate to severe CAD (17).

The present study deals with the correlation of the pre-β-1 lipoprotein with lipids and other serum and blood constituents in young males, as a pre-β-1 lipoprotein fraction appeared even in young children in the family studies (11).

Table I *Serum cholesterol and triglycerides (mean ±S.D)*

	Pre-β-1 positive (n=32)	Pre-β-1 negative (n=36)	p
Triglycerides (mmol/l)	0.89±0 40	0 75±0.31	N.S.
Total cholesterol (mg/100 ml)	178.6±36.2	157.3±24 9	$p<0.01$
Free cholesterol (mg/100 ml)	52.1±11 0	45 1±7 7	$p<0.01$

SUBJECTS AND METHODS

Seventy-five 16–17-year-old schoolboys apparently healthy at the time of the investigation, were studied The blood samples were taken at 08.00–08.30 a.m after a fasting period of 12 hours. The glass tubes were immediately capped and stored and centrifuged t 4°C Serum was used for all analyses. A SMA 12/60 (Technicon Instruments Corporation Tarrytown New York) multichannel analysis was then carried out on each sampl within 1 hour using the standard SMA 12/60 methods. Triglycerides were determined according to the method of Cramp and Robertson (4).

One tube was immediat ly placed in ice and centrifuged for 10 min at 4°C Lipid extraction was then immediately carried out Total and free serum cholesterol concentrations were determined in 68 samples using the method of Abell et al (1). The procedure and all were the same as in the proposed method ex that gas chromatographic technique was used for evaluation (6) For determination of free cholesterol hydrolysis step was omitted. A Varian Aerograph ..es 1700 (Walnut Creek Calif) was used with flame ionization detector and a stainless steel column (1/18 inch by 3 feet long and packed with 3% SE 30 on 100–120 mesh Varaport 30). 5-α-cholestane (Sigma Chemical Company) dissolved in chloroform methanol (100+120 vol) was used as internal standard Peak height measurements were sed for evaluation.

Seven hematological parameters were determined on each subject on EDTA blood with Coulter Counter Model S. (Coulter Electronics Limited Dunstable Beds. England)

Lipoprotein electrophoresis was performed by two separate methods. Cellulose acetate (Sepraphore III) lipoprotein electrophoresis, stained according to Kohn was performed as previously described (7) The agarose gel electrophoretic method was a slight modification of that described by Papadopoulos et al. (22), utilizing 0.5% agarose (5) Gel bridges were made as for protein electrophoresis (18). After levelling, a 0.5% agarose solution (Behringwerke) was poured out to produce a 1 mm thick layer on the glass plate The lipoprotein bands were stained with Sudan Black B

Statistical analysis was done using Student s *t*-test (two-tailed) for independent means.

RESULTS

A well separated pre β-1 lipoprotein fraction detectable with the eye was found in 32 of the 75 examined boys. In some cases a pre-β-1 lipoprotein fraction was found with only one of the electrophoretic methods. The reasons for this discrepancy are under investigation The agarose method seems to be more sensitive in this investigation on subjects with generally low lipid values Only one subject had a detectable pre-β-1 lipoprotein fraction on cellulose acetate which could not be verified on agarose gel

The sample of the population investigated was divided into a pre-β-1 positive ($n=32$) and a pre-β-1 negative group ($n=43$) The pre-β-1 positive subjects had a detectable fraction with both methods in 22 cases and in 10 cases only with one of the electrophoretic techniques The pre-β-1 negative subjects had no detectable pre-β-1 lipoprotein fraction with either of the two methods This classification was considered justified as the same significant differences were found if only one of the electrophoretic methods was used to classify pre-β-1 positive subjects

Plasma lipids (Table I) Higher mean values were found for both free ($p<0\ 01$) and total ($p<0\ 01$) cholesterol in the pre-β-1 positive group compared to the mean values in the pre-β-1 negative group No significant difference in mean cholesterol esterification was found between the two groups the values being 70 8% in the positive and 71.3% in the negative group

Serum electrolytes and some other serum constituents (Table II) The total CO_2 mean value was higher ($p<0\ 02$) and the chloride value lower ($p<0\ 05$) in the pre-β-1 positive group than in the group without pre β-1 lipoprotein

Hematological tests (Table III) For none of the tests were the differences in mean values between the two groups statistically significant The mean values were however higher for hematocrit, Hb RBC and MCV in the pre-β-1 positive group

Values for the red cell parameters total CO and chlorides are given in Table IV for the six subjects with the most prominent pre-β-1 fractions on both cellulose acetate and agarose Compared with pre-β-1 negative subjects differences were obtained for total CO_2 ($p<0\ 001$) chlorides ($p<0.05$) and hematocrit ($p<0.05$)

Table II. *Serum electrolytes and some other serum constituents (mean ±S.D.)*

	Pre-β-1 positive (n=37)	Pre-β-1 negative (n=43)	p
Sodium (mEq/l)	140.7±1._4	140.6±1.16	N.S.
Potassium (mEq/l)	4.1_±0.29	4.09±0.25	N.S.
Chloride (mEq/l)	101.6±1.56	102.4±1.81	p<0.05
Total CO_2 (mEq/l)	27.0±1.25	26.3±1.10	p<0.0?
Total protein (g/100 ml)	7.55±0.46	7.48±0.42	N.S.
BUN (mg/100 ml)	13.9±3.07	13.8±_.37	N.S.
Calcium (mg/100 ml)	9.94±0.29	9.91±0.35	N.S.
Alk. phosphat. (U/l)	146._±87.52	125._±50.95	N.S.
Albumin (g/100 ml)	4.55±0._4	4.57±0.23	N.S.
Uric acid (mg/100 ml)	5.52±0.77	5.57±0.97	N.S.
Creatinine (mg/100 ml)	0.89±0.14	0.91±0.13	N.S.
Bilirubin (mg/100 ml)	0.69±0.29	0.67±0.25	N.S.

DISCUSSION

Higher mean values for total and free cholesterol were found in the pre-β-1 positive group. In two recent studies (6, 15) of samples of middle-aged male patients the differences between mean cholesterol values in the pre-β-1 positive and negative groups were almost twice as high as in this investigation. In a sample of male patients, mean age 58 years, a lower mean cholesterol esterification ($p<0.05$) was also found in the pre-β-1 positive subjects (6). It is of interest that Rutenberg et al. (14, 26) found a lower cholesterol esterification in patients with acute or chronic coronary heart disease (CHD) compared to age-matched and young controls.

Studies in progress indicate that the pre-β-1 lipoprotein is a high density lipoprotein highly associated with the lipoprotein possessing the Lp( ) antigen ($0.0005<p<0.001$) (2). A lengthy discussion of the discrepancy between the two electrophoretic methods used is outside the scope of this presentation. It may depend on methodological (for example different staining techniques) as well as on other reasons. Subtypes may exist as has been suggested for the Lp( ) system (1).

The risk of CHD does not start at a certain borderline of plasma lipid but seems to increase with growing lipid levels (16, 19, 25). A higher cholesterol level in subjects with pre-β-1 lipoprotein fraction, probably existing since childhood, may be a contributing risk factor for coronary atherosclerosis.

The reason for a higher total CO_2 and lower chlorides in the pre-β-1 positive group and a higher hematocrit value for the six subjects with the most prominent pre-β-1 fraction is not obvious. A high hematocrit value has been indicated to be a risk factor for developing myocardial infarction (7). Kuo et al. (1) observed reduced saturation of oxygen in the arterial blood in 1/3 of their patients with hyperlipidemia. Hypothetically all the divergences found may primarily depend on a difference in lipoprotein composition in the pre-β-1 subjects even in the membranes, which may render the lung alveolar gas diffusion more difficult. These findings may then in part explain the higher incidence of angina pectoris in middle-aged males with pre-β-1 lipoprotein fraction.

It remains to be seen whether the predictions of this hypothesis can be verified in further studies.

Table III. *Hematological tests (mean ±S.D.)*

	Pre-β-1 positive (n=37)	Pre-β-1 negative (n=43)	p
Hb (g/100 ml)	15._±0.84	15.0±0.87	N.S.
RBC (mill./mm³)	5.05±0.26	5.01±0.31	N.S.
Hct (vol.%)	44.25±_.27	43.71±_.58	N.S.
MCV (fl)	85.35±3.63	84.78±_.33	N.S.
MCHC (g/100 ml)	34.59±0.96	34.56±0.8_	N.S.
MCH (pg)	79.43±1.47	79.41±1.07	N.S.
WBC×10³/mm³	5.79±1.30	6.11±1.17	N.S.

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Table IV. *Red cell parameters, total CO and chlorides for subjects with the most prominent pre-β-1 fraction and pre-β-1 negative young males (mean ±S.D.)*

	Pre-β-1 positive (n=6)	Pre-β-1 negative (n=43)	p
Hb (g/100 ml)	15.50±0.55	15.02±0.88	
RBC (mill./mm³)	5.22±0.22	_.01±0.31	
Hct (vol.%)	46.08±1.93	43._1±_.38	p
Total CO_2 (mEq/l)	28.00±0.49	26.29±1.10	
Chloride (mEq/l)	100.83±[illegible]	102.39±1.81	p

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EFFECT OF BICARBONATE AND PHOSPHATE ON THE ADRENERGIC RECEPTOR RESPONSE IN HUMAN ADIPOSE TISSUE IN VITRO

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Abstract The adrenergic receptor response of human adipose tissue has been tested in vitro in the presence and absence of bicarbonate and phosphate. It could be shown that omission of these ions from the medium resulted in an enhanced α-adrenergic response of the tissue. The effect of HCO_3^- was inhibited by Diamox. It is concluded that the adrenergic response is sensitive to the presence of these ions and it is suggested that it could be due to a disturbance of the calcium uptake into the mitochondria.

Human adipose tissue contains two kinds of adrenergic receptors with opposite actions on lipolysis (4) Thus stimulation of the β-adrenergic receptor increases the rate of lipolysis while α-adrenergic agonists inhibit both basal and stimulated lipolysis. The mode of action of the α-receptor in this tissue has not been clarified but several observations indicate that stimulation of the receptor inhibits the formation of cyclic AMP (6, 9) The α-receptor responsiveness of the tissue can vary and is markedly enhanced in adipose tissue from hypothyroid subjects (10 11 15) A similar enhancement of the α-adrenergic response was recently observed in normal adipose tissue specimens incubated in a sodium-deficient medium which permitted calcium to accumulate intracellularly (13).

The object of the present series of experiments was to study whether lack of ions that are of importance for the mitochondrial uptake of Ca^{++} (1 7) would change the adrenergic receptor response.

METHODS

Subcutaneous adipose tissue was obtained during laparotomy The patients did not have any malignant or metabolic disorders or jaundice nor did they take any medication. Anaesthesia was induced by a short-acting barbiturate (Nembutal®) and was maintained with Halothane® nitrous oxide and oxygen. The tissue was transported in 0.9% NaCl at 37°C from the operating theatre The adipose tissue was then cut into 50–100 mg pieces and preincubated for one hour at pH 7 4 at 37°C in the same buffer as used in the incubation except that it contained only 1 mg/ml albumin and no glucose. Separate specimens were then transferred to 1.5 ml medium containing 1 mg/ml glucose and 3 mg/ml albumin. The incubation was maintained for two hours at 37°C using air as the gas phase. The composition of the media used is shown in Table I. The release of glycerol was used as an index of lipolysis and assayed according to Chernick (3).

The following agents were used. l-noradrenaline bitartrate (Astra) phentolamine Regitine® (Ciba). N^6-2'O-dibutyryl-3',5'-adenosine monophosphate (dibutyryl cAMP) (Boehringer/Mannheim) acetazolamide (Diamox®) (Lederle).

RESULTS

The effect of the regular KHB media and the NACl–Tris medium lacking HCO_3 and PO_4^{3-} on the response to different lipolytic agents is shown in Table II The basal release of glycerol was significantly lower ($p<0.001$) in the Tris medium. The lipolytic response to 2×10^{-6} M noradrenaline was also significantly lower in the NaCl–Tris buffer both when the absolute values ($p<0.005$) and when the net responses ($p<0.025$) were compared The addition of 5 μg/ml phentolamine abolished the difference in noradrenaline response between the two kinds of buffers Phentolamine alone did not significantly modify the basal release of glycerol in either group The mean age of the subjects in the KHB group was not significantly different from that of the NaCl–Tris group (44.8 ±

Table I *Ionic composition of the buffers used (mM)*

	Na	K	Mg	Ca^{++}	Cl^-	HCO_3^-	PO_4^{3-}	SO^{4-}	Tris
Krebs-Henseleit-bicarbonate (KHB)	164	6.1	1 4	2.5	129	25	1 4	1 4	–
NaCl–Tris	160	4 6	1	1.5	169.6	–	–	–	
$NaCl–HCO_3–Tris$	160	4 6	1	1.5	140	25	–	–	
$NaCl–PO_4^{3-}–Tris$	160	4 6	1	1 5	164 6	–	5	–	2
$NaCl–HCO_3$, PO_4^{3-} Tri	160	4 6	1	1.5	135	25	5	–	2

2 8 and 37 4±3.5 years mean ±S E.M) nor was there any difference in percent of ideal body weight (16) between the KHB and NaCl–Tris group (102 4±5 0 and 101 0±3 8% mean ±S E.M)

When the net lipolytic response to 2×10^{-6} M noradrenaline was calculated as percent of that obtained in the presence of 5 μg/ml phentolamine it was 49 9±7 7% in the KHB group and 19 8±3.9% (mean ±S.E M) in the NaCl–Tris group The difference between the two groups was highly significant ($p<0\ 005$)

In order to test whether the observed differences between the two media could be accounted for by the lack of HCO_3 or PO_4^{3-} the effect of these ions was further investigated (Table III). The supplementation with 25 mM HCO_3^- slightly reduced the basal release of glycerol However neither HCO_3 or PO_4^{3-} had any significant effect on basal lipolysis when tested by analysis of variance (8) In the presence of HCO_3^- or PO_4^{3-} enaline caused a significant ($p<0\ 05$) on of lipolysis and this was further in- when both anions were added together <0 01) The responses to noradrenaline plus phentolamine were of the same magnitude in the presence or absence of PO_4^{3-} or HCO_3^- + PO_4^{3-} The response to dibutyryl cyclic AMP was slightly but not significantly increased in these two latter buffer combinations In all buffer combinations the responses to either noradrenaline in the presence of phentolamine or dibutyryl cyclic AMP were of the same magnitude and did not differ significantly when tested by paired means

When the net lipolytic response to 2×10^{-6} M noradrenaline was calculated as percent of the net response obtained with 2×10^{-6} M noradrenaline in the presence of 5 μg/ml phentolamine the results indicate that the response was significantly increased by HCO ($p<0\ 05$) alone or in combination with PO_4^{3} ($p<0\ 02$) while PO_4^{3-} alone did not have any effect (Fig 1)

Diamox® an inhibitor of the carbonic anhydrase enzyme was added to the medium in order to further investigate the effect of the HCO_3 on the adrenergic response. In this series of experiments the response to noradrenaline+phentolamine was significantly reduced ($p<0\ 05$) by HCO_3 while the response to noradrenaline alone was unaffected (Table IV) The addition of 0 45 mM Diamox® abolished the difference in response to noradrenaline+phentolamine (Table IV) This was also apparent when the lipolytic effect of

Table II *Effect of KHB and NaCl–Tris on the lipolytic response to 2×10^{-6} M l-noradrenaline in presence and absence of 5 μg/ml phentolamine (mean ±S.E M)*

	KHB ($N=16$)[a]	NaCl–Tris ($N=16$)[a]	Differences[b]
Basal	0 885±0 116	0 448±0 074	0.437±0 138 $p<0\ 005$
l-noradrenaline 2×10^{-6} M	1 843±0.207	1 021±0 131	0.8[illegible]2±0.245 $p<0.005$
l-noradrenaline–basal	0 958±0 129 $p<0\ 001$	0.573±0 096 $p<0\ 001$	0.384±0 161 $p<0\ 025$
l-noradrenaline+phentolamine 5 μg/ml	2.956±0.391	3 494±0.581	0.538±0.700 N.S
(l-noradrenaline+phentolamine 5 μg/ml)–phentolamine	2.161±0.310 $p<0\ 001$	3 100±0.562 $p<0\ 001$	0.939±0 642 N S
Phentolamine 5 μg/ml	0.794±0.126	0 394±0 071	0 400±0.144 $p<0.02$
Phentolamine 5 μg/ml–basal	0.091±0.066 N.S	–0.054±0 050 N.S.	0 037±0 083 N.S

The significance was calculated from the paired differences (8).
The significance between the means was calculated by Student *t*-test (8)

Table III. *Effect of 25 mM HCO⁻ and 5 mM PO⁻ on the lipolytic response to ×10⁻⁴ M l-noradrenaline in presence and absence of 5 µg/ml phentolamine and 10⁻⁴ M dibutyryl cyclic AMP (N=5)*

	NaCl–Tris	NaCl–HCO₃–Tris	NaCl–PO –Tris	NaCl–HCO PO –Tris
Basal	0.560±0.103	0.300±0 169	0.684±0.178	0.605±0.199
l-noradrenaline	1 642±0.391	1 742±0.367	1 900±0.279	2.980±0.598
l-noradrenaline–basal	1 082±0 434	1 441±0.317*	1.215±0.327*	2.375±0.483**
Phentolamine	0 431±0.267	0 284±0.113	0.503±0 077	0.839±0 159
Phentolamine–basal	0.129±0.214	−0.016±0.068	−0 181±0 1 1	0 234±0 088
l-noradrenaline+phentolamine	4.806±0 484	3.219±0 541	5.201±0 704	4 765±0 807
(l-noradrenaline+phentolamine)–phentolamine	4.376±0.655**	2.948±0 426**	4 698±0 664	3 927±0.771**
Dibutyryl cyclic AMP	4.555±0 901	4 014±0 495	6.135±0 965	6 056±1.289
Dibutyryl cyclic AMP–basal	3 995±0.932*	3 630±0.564	5.595±0 985	5.334±1 455

The *p*-values were calculated from the paired means $p<0.05$ $^{**}p<0.01$

noradrenaline was expressed in percent of that obtained in the presence of phentolamine (Fig. 2).

DISCUSSION

The present investigation has demonstrated that the adrenergic receptor response of normal human adipose tissue in vitro was significantly influenced by the ionic composition of the medium used.

Previous studies have also demonstrated that the α-adrenergic response of human subcutaneous adipose tissue is markedly changed when incubated in a sodium-deficient medium (13). The α-adrenergic response which inhibits the formation of cyclic AMP and thus lipolysis was enhanced in this type of buffer. The lipolytic effect of noradrenaline was however restored by the addition of phentolamine. A more detailed analysis demonstrated that the α-response was enhanced

Table IV. *Effect on the lipolytic espons to 2×10⁻⁴ M l-norad enaline (NA) and 2×10⁻⁴ M l-noradrenalin (NA) plus 5 µg/ml phentolamin (Ph) in presence and absen e of 25 mM bicarbonate in the NaCl Tris medium (N=5)*

					Diamox 0 45 mM		
Medium	Additions	Mean ±S.E.M	Mean diff ±S E M	p*	Mean ±S.E.M.	Mean diff ±S E M	p*
NaCl–Tri	None	0.39? ±0.052	–		0 625±0.179		
NaCl–Tris	NA	1 483±0.240	0 891±0 190	<0 01	1 409±0.323	0 784+0 433	N.S.
NaCl–T	NA+Ph	6.296±0 852	5 564±1 031	<0 01	4 692±0 425	3 810±0 478	<0 005
NaCl–Tris	Ph	0 732±0.228	0 140±0.232	N S	0 946±0 .43	0 3?1±0 145	N.S
NaCl–Tris–HCO₃	None	0 176±0 059			0 356±0 179		
NaCl–Tris–HCO	NA	1.340±0 295	1 164±0 295	<0 02	1 276±0 471	0 920+0 437	N S
NaCl–Tris–HCO₃	NA+Ph	1 963±0.546	1.67?±0.397	<0 02	3 082±0 689	769±0 653	<0 02
NaCl–Tris–HCO	Ph	0 29?±0 ?30	0 115±0 220	N.S	0.313±0 146	−0 043±0 043	N S
			0 273±0 363	N.S		−0 156+0 66.	N S
			3 890±1.379	<0 05		1 033±0 795	N S

$NA_{NaCl-Tris} - NA_{NaCl-Tris-HCO_3}$

$(NA+Ph)_{NaCl-Tris} - (NA+Ph)_{NaCl-Tris-HCO_3}$

The *p*-values wer calculated from the paired differences

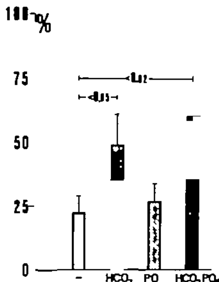


Fig 1 Effect of 25 mM bicarbonate and 5 mM phosphate on the lipolytic response to 2×10^{-6} M l-noradrenaline as calculated in percent of that induced by 2×10^{-6} M l-noradrenaline plus 5 μg/ml phentolamine The *p*-values were calculated from paired differences.

as a result of an increase in the concentration of calcium in the cells induced by the sodium deficient medium (13). So far little is known about the mechanism of action of the α-receptor in human adipose tissue However since it could be demonstrated that the hypothyroid state induced an enhancement of the α-adrenergic response in [illegible] adipose tissue (15) as well as in smooth [illegible]les from rabbit aorta (14) it was hypothesized [illegible] the mechanisms in the two kinds of tissue [illegible]uld be of similar nature (12). In smooth muscle more is known about the α-adrenergic receptor response which induces an increase in ionized calcium intracellularly which in turn causes contraction (2) The recent findings of a Ca^{2+} involvement in the α-response in normal adipose tissue would strengthen the above hypothesis (13) The object of the present investigation was to study the effect of anions known to be required for the uptake of Ca^{2+} into mitochondria (5 7) the idea being that omission of such ions would reduce the uptake of calcium into the mitochondria and thereby potentiate the α-adrenergic response It was recently shown that the uptake of Ca into mitochondria requires either PO_4^{3-} or HCO_3^- (5 7) Furthermore it could be shown that Diamox® an inhibitor of the carbanhydrase enzyme would block the effect of HCO_3 indicating that the important component of the buffer was CO_2 which was converted to CO_3^{2-} inside the mitochondrium (5) In addition to these studies Borle (1) has demonstrated that removal of PO_4^{3-} from the incubation medium causes a rapid release of Ca^{2+} from cells in tissue culture Most probably this was a release from the mitochondria in the cells. The present study has shown that removal of HCO_3 and PO_4^{3-} reduced the lipolytic response of the tissue to noradrenaline while that to noradrenaline+phentolamine remained indicating that the α-adrenergic response was enhanced by the omission of bicarbonate and phosphate The difference in noradrenaline response could not be accounted for by a difference in age or percent of ideal body weight between the two groups studied

The KHB and NaCl–Tris differ in the content of three main ions as shown in Table I The reduction of the Ca^{++} concentration from 2.5 to 1 5 mM was felt to be of less importance and could probably not account for the enhanced α-response since the experiments with La^{+++} would predict the opposite (13) The other difference was the omission of bicarbonate and phosphate in the NaCl–Tris buffer In order to further study the effect of the lack of these ions HCO_3 and PO_4^{3-}

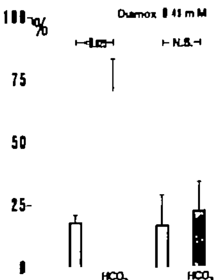


Fig 2 Effect of Diamox on the response to 25 mM bicarbonate. The response to 2×10^{-6} M l-noradrenaline was calculated in percent of that of 2×10^{-6} M l-noradrenaline plus 5 μg/ml phentolamine The *p*-values were calculated from paired differences.

were added back to the NaCl–Tris medium. The results show that the addition of HCO_3 slightly reduced the response to noradrenaline plus phentolamine while the addition of PO_4^{3-} alone or in combination with HCO_3 significantly stimulated lipolysis induced by noradrenaline both in the presence and absence of phentolamine. The response to dibutyryl cyclic AMP was similarly affected as the response to noradrenaline plus phentolamine (Table III). However when the noradrenaline response was expressed in percent of that obtained in the presence of phentolamine a significant increase was observed with addition of HCO^- alone or HCO^- and PO^{3-}. The results show that the adrenergic response is markedly influenced by these two anions and that the α-adrenergic response can be suppressed by them.

The study of Elder and Lehninger (5) showed that the mitochondrial dependence on HCO_3^- for the uptake of Ca^{2+} was inhibited by Diamox® or lack of CO_2 in the medium. This was interpreted as evidence for the entry of CO_2 into the mitochondria and conversion to HCO_3. The ion was then utilized for the trapping of Ca^{2+} inside the mitochondria. Because of these findings it was of interest to test Diamox® on the effect of bicarbonate on the adrenergic response. In these series of experiments the presence of bicarbonate reduced the response to noradrenaline+phentolamine ($p<0.05$), while the response to noradrenaline alone was not affected. Hence on a percentage basis the noradrenaline response was significantly increased, as shown in Fig. 2. The addition of 0.45 mM Diamox® markedly reduced the effect of bicarbonate while it had little effect on the response obtained in media lacking this ion. Thus it may be anticipated that the effect of bicarbonate can be antagonized by Diamox®. This would imply that it might not be the HCO_3^- per se which is active but the CO_2 which affects the adrenergic response in analogy with the results obtained by Elder and Lehninger (5). However the above noted effects of bicarbonate and phosphate should be interpreted with care and a final analysis postponed until more is known bout the intracellular compartmentalization of Ca^{2+} in this tissue.

In conclusion the present experiments ha shown that the α-adrenergic response is potentiated in medium lacking bicarbonate and phosphate. The mechanism behind this change in adrenergic response could be due to a deficient calcium buffering capacity of the mitochondria in the cells when bicarbonate or phosphate is not present in the incubation medium.

ACKNOWLEDGEMENT

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COMPARISON OF THE EFFECT OF INTRAPORTAL AND INTRAVENOUS INFUSION OF INSULIN ON BLOOD GLUCOSE AND FREE FATTY ACIDS IN PERIPHERAL VENOUS BLOOD OF MAN

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Abstract The peripheral venous hypoglycemic and hypolipacidemic responses to intraportal infusions of insulin have been compared with the responses obtained after i. infusion of identical amounts of insulin in 14 unanesthetized patients. The oute of insulin administration resulted in more pronounced hypolipacidemic response than the intraportal administration route The hypoglycemic responses, however as reflected in the peripheral venous blood, did not differ significantly after either route of insulin administration.

There is ample evidence that insulin induces hypoglycemia both by facilitating the glucose outflow from the plasma into a variety of tissues and by inhibiting the inflow of glucose from the liver to the circulation. Insulin also inhibits the adipose tissue lipolysis which according to Butcher is probably due to a lowering of the cyclic AMP level in the fat cells ( )

In a healthy subject endogenous insulin is elaborated into the portal circulation and has to traverse the liver bed before reaching the general circulation. In insulin-requiring diabetes however exogenous insulin administered parenterally reaches the peripheral tissues before passage through the liver A considerabl proportion of insulin appears to be retained during a single transhepatic passage both in laboratory animals (8 10 11 13) and man (12, 13 17) and the possibility that passage through the liver may obtund or alter the effectiveness of insul has been discussed (20) If so it is tempting to speculate about different metabolic responses which might be reflected in the peripheral enous blood after infusion of insulin via the portal vein as opposed to nfusion via a peripheral ein

The present investigation was performed on awake patients who had previously been subjected to portal vein catheterization The intention was to study the influence of the insulin infusion site on the hypoglycemic and the hypolipacidemic responses in peripheral venous blood

MATERIAL

Two women, aged 38 and 48 and 12 men aged 28–71 (mean 49), were investigated. None had glucosuria or family history of diabetes. The body weight of patient 10 was 23% in excess of the ideal body weight given in Documenta Geigy (5) The other patients were of normal body weight. Portography did not show portacaval shunts in any of the patients, but liver impairment was seen in 8 of them (Table I). A normal hospital diet was given to all the patient for at least three days prior to the investigations.

METHODS

Analytical procedures

Blood glucose was determined enzymatically with commercial glucose oxidase preparation (Kabi Reagents, Stockholm Sweden). The error of the method calculated from 15 duplicate fasting determinations according to the formula $\sqrt{\Sigma d^2/2n}$ was ±1 2 mg/100 ml (d = difference between two assays = number of duplicate assays). Free fatty acids (FFA) in serum were assayed according to colorimetric method as described by Duncombe (6) A commercially vailable kit (Biochemica Test Combination Boehringer Mannheim, West Germany) was used In this laboratory in healthy persons without family history of diabetes, the fasting FFA content of peripheral enous blood was found to vary between 0 13 and 0 89 mEq/1000 ml of serum (mean ±S E.M =0.55±0 08 mEq/1000 ml, =11) The error of the method, calculated from 11 duplicate fasting determinations according to the above formu was ±0 04 mEq/1000 ml.

Table I *Laboratory values and diagnoses*

Case no	Sex	Bilirubin (mg/100 ml)	GOT (U/ml)	GPT (U/ml)	Alk. phosph. (U/100 ml)	Portal pressure (mm H_2O)	Diagnosis
1	♂	0.4	28	43	18.8	160	Cyst of the pancreas
2	♀	0.4	20	34	9.6	360	Carcinoma of the liver
3	♂	0.5	22	19	5 4	140	Acute pancreatitis
4	♂	0 7	25	30	5.8	140	Relapsing acute pancreatitis
5	♂	11 7	38	36	23.2	170	Carcinoma of the pancreas
6	♂	2.2	78	84	36.0	120	Carcinoma of the papilla of Vater
7	♂	0.8	10	22	7.6	90	Cyst of the pancreas
8	♂	0 7	13	20	5.2	120	Relapsing acute pancreatitis
9	♂	12.5	56	40	41 0	140	Carcinoma of the papilla of Vater
10	♀	0.9	18	19	3.2	–	Cyst of the pancreas
11	♂	3 0	30	21	16.0	–	Relapsing acute pancreatitis
12	♂	0 6	62	167	23 0	100	Relapsing acute pancreatitis
13	♂	0 9	34	26	6.0	90	Acute pancreatitis
14	♂	4 0	54	51	17 0	120	Carcinoma of the pancreas
Normal values		<1.2	<40	<35	<10	<150	

Catheterizations

Portal vein catheterization was performed for diagnostic purposes (portal venography and manometry). In 13 patients (nos 1–5 and 7–14) a transumbilical catheterization technique was used (7 25) and in 1 patient (no 6) the portal vein was reached transhepatically (23 4)

Experimental model

Two experiments were made in random order on each patient. During one experiment glucagon-free insulin (Insulin Vitrum Stockholm, Sweden), 0.05 U/kg b.wt nos. 1–8 and 0.02 U/kg in nos. 9–14 was infused a constant rate for 30 min into the portal vein ng the other experiment the same amount was in- d also for 30 min, into an antecubital vein In order to obtain constant infusion rates an infusion pump (Holter 908) w used. For each patient the infusions were performed on consecutive days, and at least one day after the catheterization. Blood samples for analysis of glucose and FFA were collected 4 and 2 min before and 15 30 60 90 and 120 min after commencement of the infusion The mean of the two preinfusion values was calculated These mean values are given under the heading 0 min in Table II. All blood samples were collected from an antecubital vein opposite the peripheral infusion site

Definitions

In each experiment the glucose curve was constructed representing the per cent change of the glucose concentration above or below the basal level, in response to insulin. Then the areas circumscribed by the baseline and the glucose curve were calculated for certain time intervals (Table III) In a few instances incremental areas (i.e. areas appearing above the baseline) but in most instances decremental areas (i.e areas appearing below the baseline) were recorded. FFA curves and areas were calculated in a similar way

For statistical evaluation Student s *t*-test was applied on paired observations.

RESULTS

Fasting glucose in peripheral venous blood

The blood glucose concentrations in each pair did not differ significantly before infusion of insulin as shown in Table II

Hypoglycemic response to insulin in peripheral venous blood

Mean hypoglycemic curves are shown in Fig 1 It will be seen that insulin in a dose of 0 05 U/kg/30 min caused comparable degrees of hypoglycemia in peripheral venous blood whether infused intraportally or into an antecubital vein (Fig. 1 top) This was also true for insulin infused in a dose of 0 0 U/kg/30 min (Fig 1 bottom) The differences between paired glucose areas in response to insulin infused via the two routes, were not significant as demonstrated in Table III

Fasting FFA in peripheral venous blood

The FFA concentrations in each pair did not differ significantly before infusion of insulin (Table II)

Table II *FFA (mEq/1 000 ml) and glucose (mg/100 ml) concentrations in peripheral venous blood in response to exogenous insulin*

Poi = insulin infused via the portal vein, Pei = insulin infused via an antecubital vein. G = glucose

Case no.		0 min		15 min		30 min		60 min		90 min		120 min	
		FFA	G	FFA	G	FFA	G	FFA	G	FFA	G	FFA	G
1	Poi	0.510	93.0	0.53	72	0.58	36	0.38	41	0.53	44	0.55	49
	Pei	0.490	86.5	0.34	78	0.27	65	0.24	52	0.32	52	0.48	6[illegible]
2	Poi	0.940	90.0	0.56	80	0.30	61	0.27	54	1.10	70	1.14	75
	Pei	1.140	81.5	0.81	70	0.44	68	1.03	60	1.25	63	1.47	83
3	Poi	0.415	90.0	0.50	89	0.[illegible]1	76	0.22	68	0.41	76	0.51	78
	Pei	0.290	80.0	0.22	75	0.19	63	0.18	60	0.29	68	0.37	78
4	Poi	0.920	80.0	0.66	75	0.44	65	0.33	55	0.63	65	0.[illegible]	70
	Pei	0.605	81.5	0.33	73	0.05	50	0.05	50	0.29	77	0.63	82
5	Poi	0.280	101.0	0.22	98	0.17	86	0.20	78	0.22	78	0.32	84
	Pei	0.305	90.0	0.18	62	0.15	48	0.19	35	0.31	46	0.43	56
6	Poi	0.480	80.5	0.43	81	0.25	66	0.34	69	0.47	70	0.50	77
	Pei	0.330	100.0	0.23	76	0.08	60	0.08	59	0.40	65	0.44	68
7	Poi	0.325	83.0	0.34	77	0.16	57	0.29	54	0.74	73	0.74	75
	Pei	0.400	84.5	0.32	82	0.07	46	0.07	44	0.36	68	0.67	72
8	Poi	0.520	101.0	0.41	94	0.22	89	0.25	76	0.50	86	0.49	80
	Pei	1.290	95.5	1.04	84	0.44	80	0.80	77	1.42	79	1.38	82
Paired diff.		N.S.	N.S.										
9	Poi	0.250	99.0	0.22	93	0.14	91	0.27	87	0.27	93	0.14	93
	Pei	0.220	96.0	0.15	93	0.08	88	0.12	87	0.22	90	0.[illegible]1	90
10	Poi	0.370	68.5	0.42	64	0.35	57	0.20	64	0.24	78	0.34	80
	Pei	0.390	72.0	0.37	72	0.[illegible]4	66	0.[illegible]1	69	0.35	78	0.34	78
11	Poi	0.275	71.0	0.30	70	0.20	67	0.28	65	0.35	68	0.38	71
	Pei	0.270	91.5	0.22	88	0.18	83	0.17	77	0.28	70	0.30	88
12	Poi	0.230	78.5	0.21	72	0.16	63	0.21	72	0.30	80	0.45	80
	Pei	0.500	78.5	0.38	75	0.35	69	0.32	67	0.35	74	0.38	76
13	Poi	0.300	85.0	0.25	79	0.19	70	0.16	58	0.28	67	0.31	79
	Pei	0.265	82.5	0.19	80	0.13	68	0.05	57	0.02	75	0.14	80
14	Poi	1.030	87.0	0.90	83	0.56	77	0.74	68	1.11	84	1.15	91
	Pei	0.860	85.0	0.40	80	0.26	68	0.30	57	0.69	77	0.87	87
Paired diff		N.S	N.S										

Hypolipaemic response to insulin in peripheral venous blood

A tendency to a more pronounced mean hypolipaemic response was observed after L than after intraportal administration of insulin when a dose of 0.05 U/kg/30 min was used (Fig. 2 top). This tendency was evident also after infusion of insulin in a dose of 0.02 U/kg/30 min (Fig. 2 bottom). Significant differences between paired FFA areas were obtained during the infusion period when insulin was given in a dose of 0.05 U/kg/30 min. When the lower dose was used the differences between paired FFA areas were [illegible] cant during the infusion period and 90 min afterwards as shown in Table III.

DISCUSSION

In the present investigation intraportal and intravenous infusions of insulin were compared in unanesthetized patients. Similar studies carried out on laboratory animals have yielded contradictory results. Thus some authors have reported that the administration site has no significant influence upon the hypoglycemic response (14, 18, 19, 20) whereas others have found portal infusions less

Table III *FFA and glucose areas after infusion of insulin*

Negative values denot decremental, positive values incremental areas. Abbreviations as in Table II

Case no		Area 0–15 min		0–30 min		0–60 min		0–90 min		0–120 min	
		FFA	G	FFA	G	FFA	G	FFA	G	FFA	G
1	Pol	79	−170	161	−409	−18	−567	−34	−4 196	−266	−3 696
	Pel	−230	−74	−796	−334	−235	−1 306	−3 5 1	−503	−4 07?	−3 526
	Pol	−303	−83	−1 117	−408	−3 708	−1 491	−4 030	−439	−4 605	−3 0_3
	Pel	−217	−106	−894	−336	−1 999	−981	−1 999	−1 718	−1 381	−044
3	Pol	154	−8	−63	−133	−1 509	−733	−23	−1 333	−1 913	−1 767
	Pel	−181	−47	−6_1	−_54	−1 707	−949	−2 276	−1 549	−1 86_	−1 812
4	Pol	−212	−47	−816	−235	−2 561	−987	−3 995	−1 739	−4 532	−209
	Pel	−341	−78	−1 426	−446	−4 402	−1 607	−6 677	−770	−7 512	−2 337
5	Pol	−161	−_3	−616	−157	−1 633	−723	−38_	−1 407	−2 409	−2 001
	Pel	−308	−233	−997	−817	−3_5	−2 434	−2 863	−4 084	−2 _24	−5 385
6	Pol	−78	5	−515	−176	−1 672	−611	−2 14_	−1 021	−2 110	−1 375
	Pel	−227	−180	−1 023	−680	−3 797	−1 895	−4 093	−3 035	−3 775	−4 040
7	Pol	35	−54	−307	−343	−1 231	−1 336	518	−2 040	4 349	−364
	Pel	−150	−_3	−919	−388	−3 394	−1 791	−4 782	−2 80	−3 924	−3 317
8	Pol	−199	−52	−751	−193	−395	−744	−3 231	−1 340	−3 435	−1 903
	Pel	−146	−90	−786	−302	−345	−914	−2 821	−1 534	−2 564	−005
Paired diff		<0.05	N.S	<0.02	N.S.	N.S	N S	N S	N S	N.S.	N.S.
9	Pol	−90	−46	−310	−153	−1 040	−456	−800	−779	−1 330	−912
	Pel	−_39	−23	−1 180	−109	−2 817	−375	−3 500	−611	−3 568	−800
10	Pol	81	−50	1_3	−176	−647	−477	−1 86_	−365	−2 510	96
	Pel	−38	0	−365	−62	−1 636	−250	−2 484	−115	−2 831	134
11	Pol	68	−11	−74	−64	−437	−777	−20	−468	963	−331
	Pel	−139	−79	−528	−127	−1 583	−504	−077	−1 094	−1 855	−1 504
1	Pol	−65	−62	−358	−777	−651	−482	−33	−576	1 560	−519
	Pel	−180	−34	−585	−159	−1 575	−560	−2 565	−865	−3 375	−999
13	Pol	−125	−53	−576	−238	−1 777	−979	−578	−1 774	−2 6_5	−2 199
	Pel	−212	−23	−806	−178	−2 786	−906	−3 390	−1 506	−7 486	−1 685
14	Pol	−95	−35	−579	−199	−1 636	−784	−1 933	−1 162	−1 640	−1 145
	Pel	−401	−44	−1 306	−_38	−3 330	−1 03	−4 604	−1 667	−4 880	−1 770
Paired diff		<0 01	N S	<0 01	N S	<0.001	N S	<0 00_	N S	<0.01	N S.

effecti e than peripheral in producing hypoglycemia in dogs (9 16 21 _2) These dis parate results may be due to different experimental method including the use of glucagon-contami nated insulin preparations anesthesia and surgical alteration of the vascular anatomy It may also be a result of different infusion and blood sampling techniques

It is not known in man whether comparability of the hypoglycemic response with the two ad ministration routes is a dose-dependent effect of insulin This might in fact be true since Cheng and Kalant (3) have reported that rapidly injected insulin at a dose below 0.05 U/kg causes hypoglycemia in man by decreasing the inflow of glucose from the liver to the circulation whereas higher doses cause hypoglycemia by both decreasing the inflow and increasing the outflow of glucose into various tissues Galansino et al. (9) infused insulin in various doses into either the femoral or the portal vein of mongrel dogs and followed the changes in glucose concentration in the peripheral venous blood No differences were noted between the two routes when insul n was infused in a dose of 1 U/kg/5 min, whereas significant differences were obtained when the dose

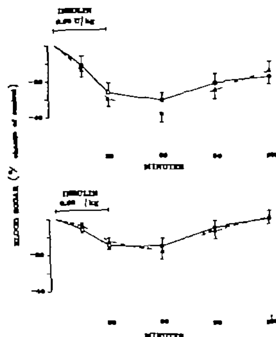


Fig 1 Comparison of the hypoglycemic effect of insulin as reflected in the peripheral venous blood, after intraportal (O—O) and i (▲ ▲) infusion of insulin (mean ±S E.M).

was reduced to 0.1 U/kg/5 min. These experiments were carried out on anesthetized animals after laparotomy Starzl et al (20) tried to avoid such disadvantages by conducting experiments on unanesthetized dogs, on which portacaval transposition had been done two or more months previously *At insulin doses of 0 0007 and 0.001* U/kg/min they found no difference in arterial hypoglycemia with the two administration sites

In the present investigation insulin was infused at slow rate because in man a greater total hypoglycemic effect has been demonstrated after a slow infusion of insulin than after a rapid injection (15) Moreover a slow infusion probably more accurately simulates the manner in which insulin is released under natural conditions Insulin in a dose of 0.05 U/kg/30 min was first given to eight subjects At this dose level the site of infusion had no significant influence upon the hypoglycemia obtained in peripheral venou blood (Table III) I order to determine whether different hypoglycemic responses with the two administration routes would appear on changing the dose of insulin, the dose initially used was reduced by 60% and the lower dose thus obtained (0 02 U/kg/30 min) was given to another six patients Neither potentiation nor obtundation of the hypoglycemic response in peripheral venous blood was obtained by primary passage of insulin through the liver at this dose level either (Table III)

Starzl et al (20) reported that the A V glucose concentration differences were not significantly different with the two routes of insulin infusion in unanesthetized dogs. Madison and Unger (14) however in comparing the intraportal and the i.v routes of insulin administration observed an increased hepatic effect (i e a decrease in the hepatic glucose output) and a decrease in the peripheral uptake of glucose of intraportally ad

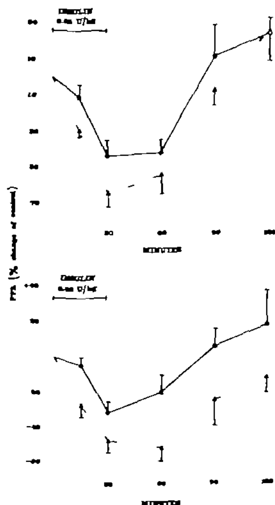


Fig 2 Comparison of the hypolipacidemic effect of insulin as reflected in the peripheral venous blood, after intraportal and infusion of insulin (mean ±S E.M) Symbols as in Fig 1

ministered insulin as compared with injection of insulin into a peripheral vein Should their results based on A V glucose gradient in anesthetized dogs, be valid also in unanesthetized man, then differences between the two administration routes might have appeared in the present investigation provided that changes in blood glucose were judged from arterial levels rather than from peripheral venous levels Moreover differences might have been forthcoming if healthy controls were tested instead of patients with various abdominal diseases.

The hypolipacidemic effect of insulin has been attributed to its ability to decrease the inflow of FFA into the circulation (1 3 4) Although an increased FFA outflow may also exert an influence upon the serum FFA level insulin does not appear to increase the outflow of FFA in man (3 4) dogs or rabbits (1)

The hypolipacidemic effect of an intraportal infusion of insulin compared with the effect of an i.v infusion of insulin has not to our knowledge been previously studied in unanesthetized subjects In the present investigation, however evidence is presented to show that insulin, infused via an antecubital vein in doses of 0.05 and 0.02 U/kg/ 30 min appears to induce a more pronounced hypolipacidemic response in the peripheral venous blood than insulin infused via the portal vein (Table III and Fig. 2)

It is well recognized in man (12 13 17) as well as in dogs (8 10 11) that a considerable proportion of insulin is retained during a single transhepatic passage Consequently an explanation of the findings presented in Table III and in Fig. 2 might be that insulin infused by the peripheral route escapes immediate hepatic retention and thus reaches the peripheral tissues at a higher concentration than after intraportal administration If so our present results are in accordance with dose-response studies in man showing a linear relationship between the depression of the plasma FFA and the dose of insulin injected (3)

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RADIOISOTOPE RENOGRAPHY GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW IN DONORS WITH NORMAL RENAL FUNCTION AFTER NEPHRECTOMY

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Abstract Radioisotope renography with [131]I-hippuran has been undertaken in 14 kidney donors with normal renal function 26–53 months after unilateral nephrectomy Immediately after renography simultaneous creatinine [125]I-iothalamate and [131]I-hippuran clearances were determined. Ratios calculated w thin the first 2.5 min of the renogram could not be correlated to clearance values or to the size of the remaining kidney There was statistically significant correlation between clearance values and renal size The range of the ratios calculated as 5–10% wherea the range of clearance values was 15–30% [131]I-hippuran reached the bladder after minimum of 2.0 min There was correlation between transit time and time to the renogram maximum.

Radioisotope renography was introduced in 1956 with tagged diodrast as a semiquantitative method for evaluation of renal function (15 18) Since the development of [131]I-hippuran by Tubis et al (16) in 1960 this technique has been widely employed Radioisotope renography presents a graphic registration of the course of activity over the kidneys after injection of a tagged material which is excreted by the kidneys—usually hippuran.

The renogram is usually divided int phases I II and III (17 19) Phase I is the first rapidly rising part of the curve and expresses the activity over the large essels. Phase I ends w thin the first minute and the curve continues with less marked rise, phase II which represents a continuing accumulation under the collimator After a maximum is reached the descending part of the curve phase III begins as radioacti ity decreases in the registration field of the collimator

Isotope renography can be used as a qualitative test of renal function (5 17 19) Pathological changes in renal function and outflow will cause changes in the appearance of the renogram (3 7 17 19) During the last five years attempts hav been made to determine glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) on the basis of phase II of the renogram (1 4 6 10 11 1)

In the present study phase II of isotope renograms obtained in subjects with one kidney and normal renal function were related to renal ize and clearance values measured immediately after isotope renography

MATERIAL

Fourteen donors were studied 26–53 months after nephrectomy Prior t donation all had normal renal func tion [125]I-iothalamate learance aried from 60 to 128 ml/min (mean 9?). [131]I-hippuran clearance varied from 301 to 650 ml/min and averaged 449 ml/min Concentration ability normal as were renal arteriograms; none had proteinuria All were normotensive and had normal fasting blood sugars Normal parameters were also found afte donation Other data are given in Table I.

METHODS

Measuring apparatus

The measuring apparatus consisted of four scintillation crystals (3 3) placed in lead collimator of cylindrical form with lumen of 3 (8.3 cm) and with 1.5 cm thick walls. The crystal ere recessed

Table II *Results of kidney function studie*

Donor no	Functioning kidney UR (2.0)	UR (2.5)	A_d/A_0	$C_{^{131}I\text{-}H}$	$C_{^{125}I\text{-}Ioth}$	C_{Cr}	Absent kidney UR (2.0)	UR (2.5)
48	1 43	1 49	1 99	328	80	107	1 11	0 99
73	1.29	1 44	1 99	470	101	104	0.84	0 81
77	1.38	1 46	1 73	255	70	62	0 95	0.95
78	1.33	1 47	2.03	282	95	111	0.85	0 79
79	1.37	1 49	1 74	352	100	98	0.87	0 87
81	1.28	1 49	1.80	448	98	102	0.88	0 85
86	1.33	1 44	1.67	372	100	102	0.97	0 97
88	1.33	1 46	1 77	200	65	75	0 89	0.80
89	1.27	1 43	1.93	170	62	66	0.86	0.86
91	1.28	1.39	1.67	283	84	85	0 85	0 85
94	1 18	1.24	1.34	202	46	73	0.78	0 72
95	1.39	1.52	1.82	269	77	77	0.86	0.87
109	1.37	1.52	2.02	317	67	90	0.85	0 85
119	1.35	1.37	1 69	212	64	79	0.89	0.89
Average	1.33	1 44	1 80	297	79.2	87 9	0.89	0 86
S.D	0 06	0.07	0.19	90.9	17 62	16.18	0.08	0 07
S.E.M	0 02	0 02	0.05	24.3	4 71	4.33	0 02	0.02

to 13 8 cm There was a positive statistically significant correlation between renal area and ^{131}I-hippuran, ^{125}I-iothalamate and creatinine clearance ($0.01 > p > 0.005$) The relationship between kidney size and ^{131}I-hippuran clearance is given in Fig 1 There was no correlation between uptake ratios and renal area.

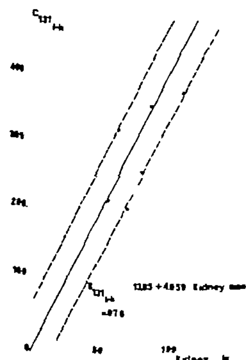


Fig 1 $C_{^{131}I\text{-}hippuran}$ ($C_{^{131}I\text{-}H}$) compared with kidney size. —fitted regression line ---±S D

Over the absent kidney UR (2.0) varied from 0 78 to 1 11 (mean 0.89 ± 0.08) UR (2.5) varied from 0 72 to 0 99 (mean 0.86 ± 0.07) ER (15) varied from 1 16 to 1 86 (mean 1.53 ± 0.17) (Table III)

The transit time was found to be between 2 and 6 min (mean 4.25 ± 1.45) Minute outputs were from *1 0 to 8 0 ml* (*mean* 3.75 ± 2.32) There was no correlation between the above mentioned parameters and ER (15)

T_{max} varied from 2.5 to 6 0 min (mean 3.59 ± 0.93) A positive correlation was found between transit time and T_{max} ($0.005 > p > 0.001$) Regression analysis and *r*-values are given in Fig. 2 ER (15) over the absent kidney varied from 1 07 to 1 53 (mean 1.27 ± 0.12).

DISCUSSION

In 14 persons with normal renal function, studied 26–53 months after unilateral nephrectomy we found no correlation between ratios calculated on the basis of the first 1.0–2.5 min of the renogram and GFR and ERPF The range of UR (2.0) and UR (2.5) values was 5% and of A_d/A_0 10% Clearance values ranged from 15 to 30% Thus it was not possible to obtain quantitative measurements of renal function from isotope renograms. Close correlation was [illegible] kid-

Table III. *Results concerning phase III of the radioisotope renogram*

Donor no.	Functioning kidney ER (15)	T_{max} (min)	Transit time (min)	Urine volume (ml/min)	Absent kidney ER (15)
48	1.53	4.1	5.0	5.8	1.15
73	1.86	4.1	6.0	2.2	1.34
77	1.43	2.5	3.5	1.6	1.21
78	1.16	3.6	5.0	1.0	1.53
79	1.65	3.0	4.5	6.4	1.27
81	1.63	4.5	4.5	6.4	1.27
86	1.52	3.9	3.0	1.1	1.07
88	1.43	2.9	3.0	5.1	1.22
89	1.75	3.7	4.0	5.0	1.31
91	1.55	6.0	7.5	1.6	1.38
94	1.53	3.3	5.0	[illegible]7	1.44
95	1.51	2.7	2.0	8.0	1.25
109	1.38	3.5	4.0	2.0	1.19
119	1.42	2.5	2.5	3.6	1.19
Average	1.53	3.59	4.25	3.75	1.27
S.D.	0.17	0.93	1.45	2.32	0.12
S.E.M.	0.05	0.25	0.39	0.62	0.03

ney size and ^{131}I-hippuran clearance. There was also a close correlation between transit time and T_{max} but no correlation between urine minute volumes and transit time with outputs in the range of 1.0–8.0 ml/min. As can be seen in Table III, activity in the bladder appeared very early during the course of the renogram (transit time minimally 2.0 min). Since the minimal time to T_{max} was 2.5 min, the ratios describing accumulation of ^{131}I-hippuran in the kidney must be calculated within the first 2.5 min of the course of the renogram.

To what degree one can use the isotope renogram to obtain a quantitative expression of kidney function corresponding to creatinine, ^{125}Iothalamate and ^{131}I-hippuran clearances is debatable. Aurell et al. (1), using A_2/A_6 in patients with normal kidney function and varying degrees of renal insufficiency not due to obstruction, found a close correlation between A_2/A_6 and C_{PAH} and

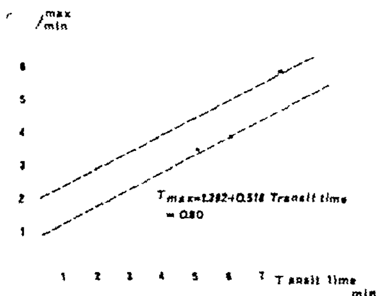


Fig. 2. T_{max} compared with transit time. Symbols as in Fig. 1.

C_{in}. Norman (11) was able to calculate ERPF with the help of plasma activity and renograms obtained by means of a γ-camera. Similar studies have been undertaken by Hirakawa et al (4) It appears possible with the use of technically complicated equipment and measurement of plasma activity to combine renographic studies with de termination of GFR and ERPF With the conventionally used simple and rapid renographic technique for recording of renograms and cystograms it has not been possible in our study on kidney donors to correlate calculations from the renogram to clearance determinations

The size of the uptake ratios calculated varied little from person to person. An explanation might be that the collimator had not covered the remaining kidney But kidney position was out lined on the basis of arteriograms previously obtained with the donors in the same supine position. The visual field should thus cover the known kidney size but it must be stressed that the actual kidney size after hypertrophy is unknown. Within the same range of clearance values the ratio A_d/A_e is found to be in the same range as reported by Aurell et al (1)

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EFFECTS OF DOPAMINE ON HEMODYNAMICS AND RENAL FUNCTION

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Abstract. I 10 patients hemodynamics and renal function have been examined before and during I infusion of dopamine Oxygen consumption was unchanged but cardiac output increased significantly on account of significant reduction of arteriovenous oxygen difference during infusion. On an average cardiac output rose by significant increase in heart rate and a slight rise in stroke volume. The pressures in the pulmonary artery wedge position and end-diastolic pressure in the left ventricle were essentially unchanged, but the systolic pressure in the left ventricle increased significantly Systemic arteriolar resistance fell significantly pulmonary arteriolar resistance revealed trend to reduction. A very significant increase in dp/dt was found. Renal extraction of PAH fell slightly PAH clearance increased significantly while the clearance of inulin showed only a minimal rise Renal plasma and blood flow and the excretion of sodium in the urine, all increased significantly There was a slight rise in urine flow and reabsorption of sodium. The results indicate that dopamine may hav favourable effect in shock, also in patients with oliguria.

Dopamine (3 4-dihydroxyphenylethylamine) is a naturally occurring catecholamine It is the biochemical precursor of noradrenaline and differs structurally from that amine only in its lack of a β-OH group (3 8)

Dopamine acts on β-adrenergic receptors to increase myocardial contractile force and heart rate This action appears to be due both to direct receptor stimulation and to secondary release of noradrenaline from nerve terminal storage sites It may increase myocardial contractility without increasing heart rate when administered intravenously to anesthetized vagotomized dogs in doses of 4–18 μg/kg (7) In larger doses tachycardia is observed At the highest doses studied (32–64 μg/kg) marked increments in heart contractile force heart rate and arterial pressure wer observed (13). Human studies revealed that heart rate either did not change or increased by less than 10 beats/min (1 7) Dopamine causes vasodilatation of the renal and mesenteric vascular beds by a nonadrenergic mechanism (5 7) It is not blocked by either α- or β-adrenergic blocking agents. The mechanism by which dopamine decreases renal vascular resistance is unknown. This latter action has not been observed with other β-adrenotropic receptor stimulators and is unique for catecholamines (20) Dopamine should be especially suitable in treatment of shock (1 9 18). Further investigations of the effects of dopamine would therefore be of interest.

MATERIAL AND METHODS

The material comprised 10 patients 5 women and 5 men, of verage age 43 years. They had all primary myocardial disease

Right heart catheterization was performed with Cournand catheter A polyethylene catheter with side splits was placed by the Seldinger technique in the aorta and left ventricle via the femoral artery A green Oedman catheter was introduced by the Seldinger technique via the femoral vein into the right renal vein. The urinary bladder was also catheterized Pressures were recorded in the pulmonary artery wedge position, left ventricl and aorta In addition dp/dt was recorded from the left ventricle. Cardiac output was measured according to Fick principle, using Douglas bag Oxygen consumption was calculated from gas analyses, carried out by the micromethod of Scholander

The renal function was studied by examining enal clearance of inulin and para-aminohippuric acid (PAH) renal extraction of PAH renal plasma flow and blood flow excretion of sodium in the urine and tubular reabsorption of sodium and diuresis.

A solution of 0.2 mg dopamine in 1 ml 5% glucose was given intravenously The dose was approximately 4 μg/min/kg After infusion for 15 min the cardiorenal hemodynamic studies were repeated. The clearances were examined in two periods i all patients before infusion of dopamine During infusion new pe-

Table I *Hemodynamic effects of dopamine*

	No. of pats.	Before		After			
		Mean	S D	Mean	S.D.	*t*	*p*
Heart rate (beats/min)	10	77	26	90	36	2.45	0 025<p<0.05
Mean pulmonary capillary wedge pressure (mmHg)	10	17	7	17	9	0.09	–
Mean pulmonary arterial pressure (mmHg)	10	25	10	26	10	0 80	–
Systolic left ventricular pressure (mmHg)	10	137	4	152	29	4.38	0 001<p<0 005
LVEDP (mmHg)	10	15	6	15	9	0.57	–
Diastolic aortic pressure (mmHg)	10	86	15	76	17	3 16	0.01<p<0 02
Mean aortic pressure (mmHg)	10	104	15	94	18	3.29	0.005<p<0 01
Oxygen consumption (ml/min)	10	308	86	309	53	0 05	–
Arteriovenous oxygen difference (ml/l)	9	51 1	16.8	37 6	10 4	5 49	0 001
Cardiac output (l/min)	9	6.9	3 4	8.8	2.5	3 1	0 01<p<0 02
Cardiac index (l/min/m²)	9	3 7	1.6	4 7	1.2	3.36	0 005<p<0 01
Stroke volume (ml)	9	96	59	112	69	1.68	–
Pulmonary arteriolar resistance (dyn/sec cm⁻⁵)	9	121	84	97	56	1 72	–
Systemic arteriolar resistance (dyn/sec cm⁻⁵)	9	1 400	562	946	308	3 65	0.005<p<0.01
dp/dt (mmHg/sec)	10	309	639	4 002	1 287	6 47	<0 001
T-peak dp/dt (sec)	10	0 097	0 000	0 070	0 000	3.86	0 001<p<0 005
LVSW (g-m)	9	121.0	79 1	131.8	91 6	0 77	–

tients were examined in two periods, but one patient on account of tiredness in only one period.

The patients were informed of the purpose of the study and had given their consent.

RESULTS

The data recorded are presented in Tables I and II.

During infusion of dopamine a significant increase in cardiac output was observed (Fig. 1). The oxygen consumption was constant, but the calculated cardiac output increased on account of a significant reduction in arteriovenous oxygen difference (Fig. 2). There was a significant increase in heart rate (Fig. 1). On an average the increment was 13 beats/min. In one patient the heart rate rose from 86 to 143 and the dose of dopamine was reduced. In most of the patients the rise was moderate. Two patients had nodal bradycardia beforehand and the rate increased only from 38 to 40 and from 46 to 50. The stroke volume increased slightly, on an average from 96 to 112 ml (Fig. 1). Consequently the cardiac output rose by a significant increase in heart rate

Table II *Effects of dopamine on renal function in 10 patients*

	Before		After			
	Mean	S D	Mean	S D	*t*	*p*
Renal extraction of PAH (%)	87	5	85	7	1 18	
Renal plasma flow (ml/min)	459	116	680	790	3 19	0 01<p<0 02
Renal blood flow (ml/min)	779	182	1 154	473	3.28	0.005<p<0 01
PAH clearance (ml/min)	393	93	565	210	3 41	0 005<p<0 01
Inulin clearance (ml/min)	114	23	105	23	1.26	
Sodium reabsorption (mEq/min)	14 08	3 15	15 09	3 47	1 02	
Urine flow (ml/min)	4 1	3 4	6.7	3 6	2.07	–
Excretion of sodium in urine (µEq/min)	213	159	719	448	3.32	0 005<p<0 01

CO l/min — Stroke volume (ml) — Heart rate (beats/min)

15, 12, 9, 6, 3 — 250, 200, 100, 40 — 150, 120, 90, 60, 30

Dopamine Before After — Dopamine Before After — Dopamine Before After

Fig. 1. Cardiac output, stroke volume and heart rate before and after dopamine.

and a slight rise in stroke volume I the patients with nodal bradycardia there was chiefly a rise in stroke olume

The mean pressure in the pulmonary artery and in wedge position (Fig. 3) and the end-diastolic left ventricular pressure (LVEDP) were essentially unchanged (Fig. 4). The systolic left ventricular pressure increased significantly (Fig. 4) but the diastolic and mean aortic pressure fell significantly from the time before infusion until

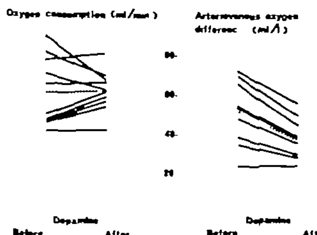


Fig. 2. Oxygen consumption and arteriovenous oxygen difference before and after dopamine

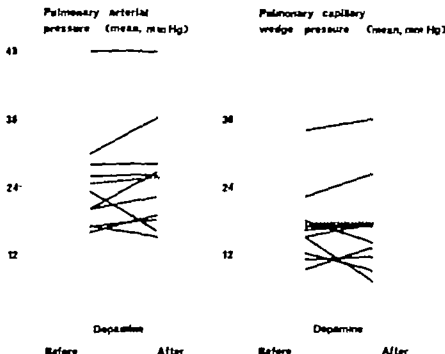


Fig 3 Mean pulmonary artery and pulmonary capillary wedge pressure before and after dopamine.

the last period of infusion (Fig. 5) The investigation also revealed a significant reduction in systemic arterial resistance There was also a slight fall in pulmonary arteriolar resistance (Fig. 6)

During infusion of dopamine dp/dt increased very significantly (Fig 4) This parameter had the highest *t* value using Student's *t*-test The time until attainment of peak of dp/dt (*t* peak dp/dt) (11) shortened in eight patients and was unchanged in two The average value decreased from 0 097' to 0 070

Plotting of left ventricular stroke work (LVSW)

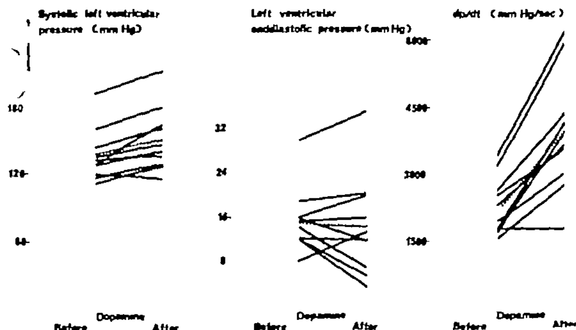


Fig 4 Left ventricular systolic and end-diastolic pressure and dp/dt before and after dopamine

Aortic Pressure (mean, mm Hg) Diastolic Aortic Pressure (mm Hg)

120 120

90 80

Borrower's No.	Due Date	Borrower's No.	Due Date

After

Fig 5 Mean and diastolic aortic pressure befor and after dopamine

while the clearance of nulin revealed only lest increase (Fig. 7) Both renal plasma and flow rose signif cantly (F g. 8) The cal- d renal vascular resistance was consequently ed The renal blood flow ncreased on an ge 407 ml/min when the body surface is aken into consideration while the cardiac

se sec cm⁻⁵)

Before Dopamine After Before Dopamine After

F g 6 Pulmonary ar teriolar (*PAR*) and ys- temic arterial esistance (*SAR*) before and after dopamine

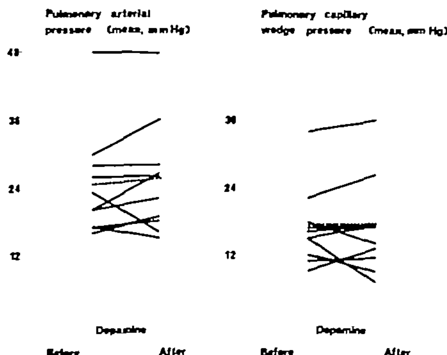


Fig 3 Mean pulmonary artery and pulmonary capillary wedge pressur before and after dopamine

the last period of infusion (Fig. 5) The investigation also revealed a significant reduction in systemic arterial resistance There was also a slight fall in pulmonary arteriolar resistance (Fig. 6)

During infusion of dopamine dp/dt increased very significantly (Fig. 4) This parameter had the highest *t* value using Student s *t*-test. The time until attainment of peak of dp/dt (*t*-peak dp/dt) (11) shortened in eight patients and was unchanged in two The average value decreased from 0 097' to 0.070'

Plotting of left ventricular stroke work (LVSW)

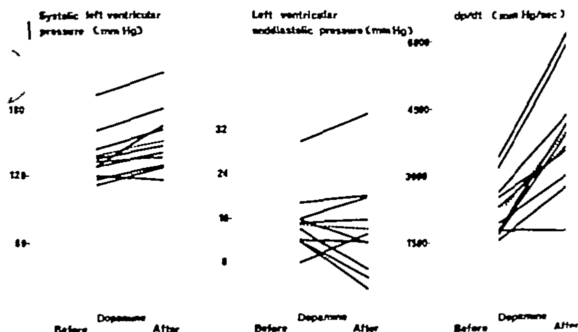


Fig 4 Left ventricular systolic and end-diastolic pressure and dp/dt before and after dopamine

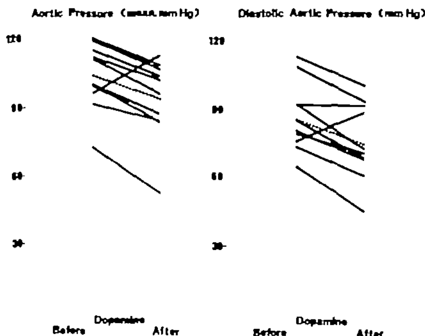


Fig 5 Mean and diastolic aortic pressure before and after dopamine

against LVEDP (Fig. 9) demonstrates rise of LVSW in 5 patients and a fall in 4 There is also a fall of LVEDP in 4 patients and a rise in 4 On an average ther is no change in left ventricular f nction curve.

There was only a slight fall in renal extraction of PAH (Fig. 7). PAH clearance increased significantly while the clearance of inulin revealed only a modest increase (Fig 7) Both renal plasma and blood flow rose significantly (Fig. 8) The calculated renal vascular resistance was consequently reduced. The renal blood flow increased on an average 407 ml/min, when the body surfac is not taken into consideration while the cardiac

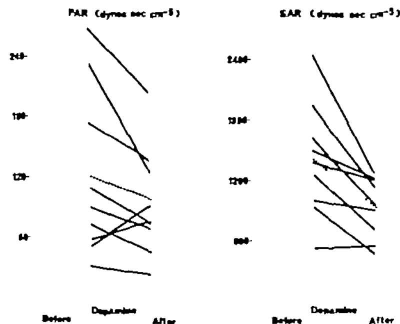


Fig 6 Pulmonary arteriolar (*PAR*) and systemic arterial esistance (*SAR*) before and after dopamine.

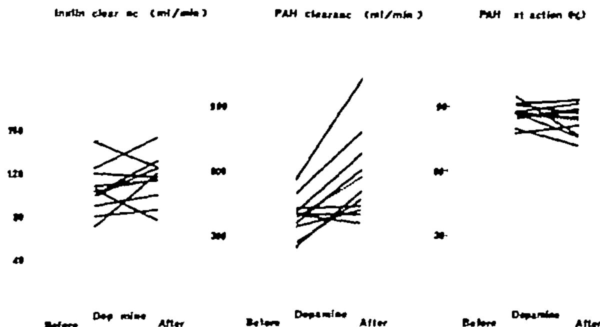


Fig 7 Inulin and PAH clearance and PAH extraction before and after dopamine.

output increased 1878 ml/min, 22% of the increase thus occurring in the renal circulation. The renal blood flow before dopamine was 1 % of the cardiac output during infusion of dopamine 14%

Dopamine caused a slight increment of diuresis but the excretion of sodium in the urine increased significantly Reabsorption of sodium rose only moderately

One of the patients had beforehand a tendency to premature beats and this tendency increased during infusion of dopamine

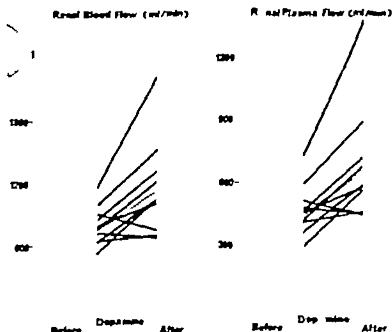


Fig 8 Renal blood and plasma flow before and after dopamine

Fig 9 Effect of dopamine on left ventricular stroke work and left ventricular end-diastolic pressure.

DISCUSSION

Different effects of dopamine and noradrenaline suggest that the effects of dopamine do not depend entirely upon conversion to noradrenaline (14) Earlier investigations reveal an increase in cardiac output (1 7 8 10 11 18 20) and renal blood flow (14 16) Other catecholamines will also increase cardiac output, but as a rule the renal blood flow is reduced (5).

In treatment of shock, therefore dopamine is a more convenient agent than other catecholamines (10 11), especially in oliguric patients with low or normal systemic resistance (7) The renal blood flow before dopamine was in this investigation 12% of cardiac output during infusion of dopamine 14% I the in estigation by McDonald et al (14) the renal fraction of cardiac index did not change significantly

In several investigations (1 8 20) there wa a relatively small or no increase in heart rate Goldberg et al. (7) found no change or a slight increase, and also others (8, 14) concluded that dopamine has less tendency than other amines to increase heart rate. Nayler et al. (19) injected dopamine directly into the coronary circulation in heart preparations and this resulted in an immediate increase of heart rate. In the present investigation there was a significant increase of heart rate Most investigators (8, 20) have found an increased stroke olume. In our patient only a slight rise was observed

Arteriovenous oxygen difference was reduced significantly while oxygen consumption was unchanged These findings confirm the results of Horwitz et al. (8) by indicator dilution technique In their investigation the calculated arteriovenous oxygen difference decreased in five of six subjects and did not change in the last one indicating that the increase in blood flow was not accompanied by a proportional increase in total body oxygen demand Rosenblum et al. (20) measured the cardiac index by Fick and/or indicator dilution methods and made the same observations

Pulmonary arteriolar resistance fell moderately Other estigators (20) found a decrease of 21% Usually an ncrease in ystolic arterial pressure was observed (2 9) as n oo material but changes n diastolic arterial pressure ha e dif fered Goldberg et al (7) found an increase de crease or no change. In the present investigation there was a significant fall in diastolic aortic pressure

In accordance with earlier studies (1 7 8 11 14) the calculated systemic arteriolar resistance was reduced. Goldberg (5) emphasizes that total peripheral resistance is not reduced to the same extent as with isoproterenol or adrenaline. Horwitz et al (8) by i v administration of dopamine to normal subjects found a decrease or no change in peripheral resistance

A distinct increase in dp/dt in the left ventricle was revealed during infusion of dopamine dp/dt is affected by alterations of arterial diastolic pressure a determinant of ventricular afterload An elevation of arterial diastolic pressure results in a rise of peak dp/dt (1). In the present material the diastolic aortic pressure fell Theoretically dp/dt therefore would have increased still more if the afterload had been unchanged. Increase in heart rate will also increase dp/dt, and consequently a part of the increase of dp/dt in this material could be caused by the rise in heart rate. Ventricular preload is augmented by the elevation of end-diastolic pressure (4) but this parameter did not change in this study It may thus be concluded that during infusion of dopamine a distinct increase in dp/dt is observed as a sign of increased myocardial contractility This is confirmed by the average decrease of *t*-peak dp/dt in the present material. Mason (1) emphasizes that, both in intact canine preparations and in conscious patients when *t*-peak dp/dt was found to shorten while peak dp/dt increased an augmentation of myocardial contractility always occurred *Increase of dp/dt has also been found by other* during infusion of dopamine (20)

At variance with the observed rise in dp/dt is absence of improvement in left ventricular function curve plotted on the Starling diagram. The changes on the diagram vary very much from patient to patient. On the whole these changes are ery similar to those observed in an earlier study on glucagon (22)

Renal extraction of PAH is a rather constant value and there was no change during the investigation. In the dog dopamine infusion may decrease renal extraction of PAH (17).

In this study a significant increase in PAH clearance renal plasma and blood flow was found corresponding to earlier studies Meyer et al (17) in water-loaded dogs observed that infusion of dopamine produced an increase in sodium potassium and osmolal excretion clearance of PAH and inulin also increased Goldberg et al (7) found that PAH clearance on an average increased from 507 to 798 ml/min while our value increased from 393 to 565 ml/min Goldberg (5) observed that dopamine increased renal blood flow about 30% while Rosenblum et al. (20) observed a 79% increase The increase in inulin clearance was only moderate in this material but other in estigators ha e found a larger increment. Goldberg et al (7) found an average increase in inulin clearance from 109 to 128 ml/min Rosenblum et al (20) an increase of 38% In this material inulin clearance increased from 105 to 114 ml/min McDonald et al. (14) found that glomerular filtration rate and renal plasma flow increased significantly in normal subjects, only slightly in patients with congestive heart failure. We found a decrease in renal vascular resistance, corresponding to earlier findings by McNay et al (16) of a 30% decrease.

Diuresis increased slightly but this parameter has increased more in other studies (10). Talley et al (?1) emphasize that the increase in urine flow produced by dopamine in the majority of patients suggests that the renal vascular bed is not adversely constricted by dopamine The excretion of sodium in urine was significantly increased and the same observations are reported by others both in dog and man (1 7 14) When dopamine is administered directly into one renal artery in dog at a rate of 0 6 μg/kg/min the infused kidney shows a greater increase in sodium excretion than the contralateral kidney (7 17) A vasodilatation of the renal vessels is proposed as a possible cause (10 11 15 16) Examinations on canine renal and femoral blood flow confirmed the ability of dopamine to dilate the renal vascular bed without producing a qualitatively similar direct effect on the femoral vascular bed This finding sets it apart from agents such as papa erine and the nitrites (15).

Some of the effects of dopamine are similar to that of aminophylline. McDonald et al (14) summarizing the results of other investigators state that in normal subjects and in patients with congestive heart failure aminophylline increases cardiac output glomerular filtration rate renal plasma flow and sodium excretion Adrenaline and metaraminol occasionally produce moderate increments in sodium excretion but these amines also increase the mean arterial blood pressure The

natriuresis produced by dopamine cannot be explained by such a mechanism since there was no significant change in mean arterial pressure in the investigations by McDonald et al (14). In our material there was a significant fall in mean aortic pressure

One of our patients had beforehand a tendency to premature beats which increased during infusion of dopamine It is also mentioned by others (7) that dopamine as any other sympathomimetic amine acting on β-adrenergic receptors can cause arrhythmias Goldberg et al (6) however report that frequent ventricular premature beats were observed in the ECG of each patient before dopamine infusion, and the extrasystoles did not increase during the infusions Increase of ventricular extrasystoles has previously been observed (14) In one instance ventricular tachycardia occurred during dopamine infusion (21)

In conclusion this study has confirmed earlier observations (11 18) that dopamine increases the cardiac output both by an inotropic and chronotropic effect In addition the blood flow in renal vessels is increased A favourable effect on the oliguria of shock may be expected although none of our patients were in a state of shock during the study

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RELATIONSHIP BETWEEN BLOOD PRESSURE AND PHYSICAL FITNESS SMOKING AND ALCOHOL CONSUMPTION IN COPENHAGEN MALES AGED 40–59

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Abstract The separate relationships between BP and age, physical fitness, relative weight, smoking and alcohol consumption have been studied in a cardiovascular survey of 5249 middle-aged males. Increasing age, relative weight and alcohol consumption were all positively related to the height of BP In contrast physical fitness and smoking were negatively related. The separate linear effects of age relative weight and physical fitness were estimated by means of multiple regression analysis. The BP differences between smokers and non-smokers and drinkers and non-drinkers could not be explained by differences between the groups in respect of age relative weight or physical fitness.

In prospective cardiovascular surveys it has been demonstrated that high BP is an important risk factor in coronary heart disease (33) and cerebrovascular disease (16) Furthermore it has been found that the incidence of these diseases increases linearly with BP rising from values generally considered to be low-normal (17) Factors related to the BP level within population groups should therefore be investigated in detail Although much previous work has been carried out in this field, further knowledge is needed.

In a cardiovascular survey (12) we have studied the relationship between physical inactivity and coronary heart disease In this study the BP level was found to be related to the following factors age, relative weight, physical fitness smoking habits and habitual alcohol consumption. In the present paper the separate relations between these factors and BP are evaluated.

MATERIAL AND METHODS

The sample comprised 5249 men aged 40–59 years, all employed in large private or public enterprises in Copenhagen. All men aged 40–59 employed in the enterprises were invited to participate The response rate of participation was 87.3% The methods used have previously been described and discussed in detail (12) and will be briefly summarized.

The examination of very subject comprised: 1) A short interview by one of us (F G.) on the basis of questionnaire completed beforehand 2) BP measurement. 3) Measurement of height and weight. 4) Indirect measurement of maximal oxygen uptak

Blood pressure was measured by one observer (F G) in order to avoid interindividual differences in recordings. After at least 5 min rest with the subject sitting, 12 cm broad and 26 cm long cuff was firmly and evenly applied to the subject right arm, the lower edge of the cuff being 2 cm above the antecubital pace. Diastolic BP was recorded by disappearance of the Korotkow sounds.

Indirect measurement of maximal oxygen uptake was performed on Monarch bicycle ergometer (1) with the loads 600 900 or 1200 kpm/min. Heart rate was measured during work in steady state using a stop watch and stethoscope. The load chosen in each case was determined from the age and weight of the subject or from the heart rate during the first minute of the test. In individual cases two load were used. Åstrand' nomogram was used for determination of maximal oxygen uptake. All measurements of height and weight and the Åstrand test were performed by two specially trained nurses. Relative weight was calculated from the tables of height and weight of 22000 normal Danes drawn up by the Hafnia Insurance Company

The subjects did not smoke during the last 30 min before the examination nor eat and drink during the last 1 hour

Statistical methods and processing of data

All data were transferred to punch cards and processed at the Northern European University Computing Centre. The following three analyses were carried out: cross-tabulation using χ^2-test as test of significance, multivariate analysis of dispersion, and finally multiple regression analysis. Before performing the two latter analyses data were checked for normality and, if neces-

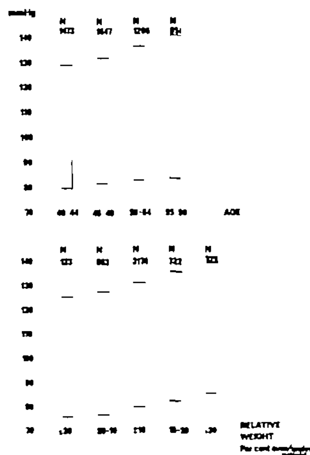


Fig 1 Mean BP at different ages and relative weights. Upper ends of the bars indicate ystolic, lower ends diastolic BP

sary transformed. Most data were logarithmically ormed the analysis of dispersion to obtain nor ty and in the regression analysis to obtain linear tions 1 the regression analysis residuals were ecked for normality and ndependen e of parameters. The multivariat and regression analyses enabled an analyses to be made of the single factors keeping the others constant

RESULTS

Blood pressu e and age relative weight and physical fitness

Mean values of systolic and diastolic BP in four age groups and for five different levels of relative weight are illustrated in Fig. 1 In Fig. the distribution of subjects within quintiles of physical fitness is seen for low and high systolic BP values The distribution with respect to diastolic BP reached the same level of statistical significance ($p<0\ 0005$) and exhibited a quite similar pattern (not illustrated).

The age distribution within the material did not allow the performance of multivariate analysis. The positive relationship between age and BP was therefore estimated by a χ^2-test ($p<0\ 0005$). The partial correlation coefficient between systolic BP and relative weight was 0.27 ($p<0\ 0005$) and between diastolic BP and relative weight 0 19 ($p<0\ 0005$) The partial correlation coefficient between physical fitness and systolic BP was −0.28 ($p<0\ 0005$) and between physical fitness and diastolic BP −0.20 ($p<0\ 0005$) Partial correlation coefficients for the relationship between BP and relative weight and BP and physical fitness were calculated for constant values of relative weight when calculating for physical fitness and vice versa

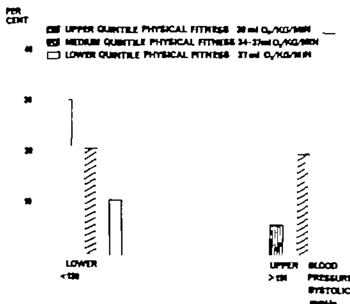
The linear effects of the above variables were estimated by multiple regression analysis. For constant levels of relative weight and physical fitness the linear effect of *age* was 0 2 for both systolic and diastolic BP Thus a decade increase in age was accompanied by an increase of 2 mmHg in both systolic and diastolic BP

For constant levels of age and physical fitness the linear effect of *relative eight* was 0.. for systolic and 0.3 for diastolic BP A 10% increase in relative weight is thus accompanied by an increase of mmHg in systolic and mmHg in diastolic BP

For constant levels of age and relative weight the linear effect of *physical f tness* on both systolic and diastolic BP was −0.2 Thus a 10 ml/kg/min increase in maximal oxygen uptake is related to ? mmHg decrease in both systolic and diastolic BP

Blood pressure and smoking habits

Mean BP values for smokers and non-smokers (ex smokers for more than one month and subjects having never smoked) are seen in Table 1 No differences in BP values were found between ex smokers and those who had never smoked, therefore the group of non-smokers is treated as a whole in tabulations Smokers had lower BP than non-smokers ($p<0\ 0005$). Mean systolic and diastolic BP values for different categories of smokers are also shown in Table 1 Cigar smokers had higher diastolic BP than other smokers ($p<0\ 0005$) but the difference from systol c BP values did not reach conventional



F g 2 Percentage distribution of physical fitness quintiles within lower and upper systolic BP quintiles

levels of significance ($p<0.1$) Cigar smokers were heavier than other smokers. Pipe smokers had lower BP than other smokers ($p<0.02$ for systolic and $p<0.0005$ for diastolic) Also smokers who inhale had lower diastolic BP than those not inhaling ($p<0.1$ for systolic and $p<0.0005$ for diastolic).

Table I. *Mean systolic and diastolic BP in different group ith respect to smoking habits*

	N	BP Syst.	BP Diast.
Smokers	3 774	133 6	82.1
Smokers of			
Cigarettes >10/d.	571	135 0	81.8
Cigars >5/d	84	139.2	85 4
Pipe >6/d	824	130.3	78 9
Cheroots >6/d.	227	133 9	80.2
Non-smokers	1 454	138 6	85.9
Inhalers	2 127	132.2	79.9
Non-inhalers	731	134.2	81 9

Table II *Mean systolic and diastolic BP in group ith different al ohol consumption according to answers to qu stionnaire*

Unfilled spaces due to observations on less than 16 subjects

	Alcohol (units/day) 0	1–2	3–5	6–10	10
N	1 374	1 930	577	92	15
BP syst.	133.5	13? 6	137 0	141 6	
BP diast.	80 9	80.9	83.0	85 7	

Alcohol consumption and blood pressure

Mean values of systolic and diastolic BP for each group of subjects according to recorded alcohol consumption per day are seen in Table II The alcoholic beverage consumed by the participants was mainly beer A statistically significant increase in both systolic and diastolic BP was found with an increased alcohol consumption ($p<0.0005$)

Smoking and alcohol consumption related to the other variabl s

In Table III are seen the mean values of BP relative weight physical fitness and age of smokers and non-smokers drinking various amounts of alcohol per day Considering the linear effects of age relative weight and physical fitness on BP the following conclusion can be reached. the differences in BP between smokers and non-smokers and between drinkers and non-drinkers cannot be xplained by the differences in relative weight or physical fitness between the groups No deviation from this pattern was observed when analysing different categories of smokers smoking various amounts of tobacco or

Table III *Smoking and alcohol consumption related to physical fitness age relative weight and BP*

Unfilled spaces due to observations on less than 16 subjects
S = smokers, N-S = non-smokers

		Alcohol (units/day)				
		0	1–	3–5	6–10	10
Physical fitness (ml O_2/kg/min)	S	33.4	33 7	32.0	30.0	
	N-S	32.8	32..	30..		
Age (y.)	S	48 0	48.4	49 0	48.6	
	N-S	48..	48 1	49 1		
Relative weight (%)	S	99.8	100 0	103.3	107.5	
	N-S	101.3	102.9	109.2		
BP syst.	S	131.2	131 6	135 4	137 4	
	N-S	136.3	136 8	141.2		
BP diast.	S	80 6	80.7	83.5	83.6	
	N-S	84.4	84 9	87.9		

different kinds of tobacco. Subjects drinking much alcohol were more likely also to smoke large amounts of tobacco ($p<0.0005$).

DISCUSSION

In a previous paper (13) it was demonstrated that physical fitness as determined here was well correlated to leisure-time physical activity In previous studies by others, physically active ..ps were found to have lower BP than more ..ntary groups (18 22, 4 27) Also the most ..ysically fit subjects found by the use of an indirect fitness test by Balke (2) had lower BP than the less fit subjects a finding quite consistent with the findings in the present study

In a few studies on the effects of physical training the BP decreased after a period of training (4 6, 11 14) a finding also suggesting a cause–effect relationship between physical conditioning and BP However the practical clinical importance of this relationship is probably only a minor one Although the data from the present study derive from a cross-sectional study the finding that a 10 ml/kg/min increase in maximal oxygen uptake is accompanied by a decrease in BP of no more than mmHg systolic and diastolic, indicates that the influence of physical fitness upon BP is actually quite small. In a sedentary middle aged male an increase of the above magnitude in maximal oxygen uptake will require a steady and intensive period of physical training. Nevertheless this relationship may be part of the explanation why high BP is rare among a population that is extremely physically fit, like the Masais (23).

The increase in BP with age found here has also been demonstrated in most previous epidemiological studies performed in industrialized countries (10) The separate influence of age upon BP in the present study was small i.e .. mmHg/decade for both systolic and diastolic BP But the actual difference between the age groups was about 7 mmHg/decade for systolic and 2.5 mm/decade for diastolic BP This suggests that part of at least the actual increase in systolic BP with age is due to increase of weight and decrease of physical fitness with age, this again being related to long-term exposure to environmental factors such as the habits of overeating and lack of exercise so prevalent in all developed countries

As already demonstrated by Boe et al. (3) although a highly statistically significant relationship between weight and BP exists the linear effect of BP on weight is small. In the present study increases of 2 mmHg for systolic and 3 mmHg for diastolic BP were found for each 10% increase in relative weight. Boe et al. (3) found exactly the same figures for each 10 kg increase in body weight. Since a standard cuff was used in the present study it is likely that the effect of relative weight on BP is overestimated. In very obese subjects with large arm circumference there is a tendency to measurement of higher diastolic BP values (29) The quantitative role of this factor is not yet quite clear (28).

Do the results of the present study and those cited have significant practical implications in the management of hypertensive patients and/or in public health? Common medical advice to the hypertensive patient is to lose weight in case of obesity and to make moderate exercise. The above discussed results lend support to this advice but in a patient with a well established hypertension and a high BP i.e. 200/115 mmHg, the therapeutic effects of these hygienic measures may be expected to be small, even assuming that the advice is strictly adhered to by the patient. In subjects with borderline hypertension, i.e. systolic BP 140–160 mmHg and diastolic 90–105 it might, however be justified to attempt hygienic measures

for a period before possible antihypertensive medical treatment is instituted Weight reduction and controlled physical training perhaps should be the first therapeutic measure for patients with small BP elevations. The results obtained by Boyer and Kasch (4) are convincing.

According to the calculations of Stitt et al (35) only a small reduction of mean BP in a whole population may mean a substantial decrease in the prevalence of hypertension Therefore the above results may be relevant for prevention of cardiovascular disease in whole populations. At least they add further support to the hypothesis that leisure-time physical activity may be a preventive measure in coronary heart disease (26). Also it is noteworthy and supports the calculations of Stitt et al (35), that only very few physically fit subjects had systolic BP values above 150 mmHg compared to the least physically fit.

The present observation that smokers have lower BP than non-smokers confirms the findings in several previous studies (9 19 24 30) The new observation is that this difference cannot be explained by differences in weight between smokers and non-smokers as has previously been claimed (19 30). Neither can the difference be explained by differences in physical fitness between smokers and non-smokers. The acute effects of smoking, both at rest and during exercise upon pulse rate and BP are similar to those of exercise causing a rise in both (15 20) In sedentary subjects therefore the long-term effects of smoking might be similar to the effects of physical training (8). Thus subjects participating in a smoking-withdrawal programme and having managed to stop smoking actually had a decrease in maximal oxygen uptake per kg body weight (31). This hypothesis may provide a reasonable explanation of the odd findings that smokers are a little more physically fit than non-smokers and have lower BP On the basis of these observations and the above hypothesis smoking might be considered beneficial in the prevention of cardiovascular disease This is, of course in direct contrast to the observations on the hazards of smoking which have now been demonstrated in numerous epidemiological studies (33). The conclusion to be drawn from the present results might therefore be that, in spite of the possible long-term beneficial hemodynamic effects of smoking, other effects arising from it make smoking a risk factor in cardiovascular disease (21). Since pipe smoking, however has not been found a risk factor in cardiovascular disease (34) in the light of the present findings that pipe smokers have lower BP than other smokers it may be of questionable value to abandon pipe-smoking. Recent results do however indicate that also pipe-smoking at least when inhaling may be unwise (5)

Like the differences concerning smoking, the differences in BP between groups drinking various amounts of alcohol could not be explained by differences in weight or physical fitness between the groups. The results of the present study and those of others (7 32) demonstrate that subjects drinking much alcohol have higher BP than those who do not drink This may indicate that subjects drinking much alcohol have an increased risk of cardiovascular disease. Up to now however alcohol has not been found a coronary risk factor (33)

CONCLUSION

From the presented data and those cited it is possible to draw a part of a profile of the middle aged mal most likely to have a high BP This man is sedentary with a low physical fitness obese a quite heavy drinker and moderate smoker or does not smoke at all.

ACKNOWLEDGEMENTS

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URINARY GROWTH HORMONE IN ACROMEGALY

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Abstract Urinary growth hormone compared to plasma growth hormone during peroral glucose tolerance test has been studied in 16 patients with clinically obvious acromegaly The urinary excretion of growth hormone was clearly elevated in all patients with acromegaly hen compared to a normal group. One of the patient with established acromegaly had normal fasting plasma growth hormone levels, but urinary growth hormone was greatly ele ated No significant correlation was found between plasma and urinary growth hormone in the individual patient. Neither plasma nor urinary growth hormone were well correlated to the clinical activity of the disease It is concluded that measurement of urinary growth hormone is valuable tool in the diagnosis of acromegaly The measurement might be of special interest in the early period of acromegaly before the disfiguring changes have taken place n the patients.

It is well known that there are rapid changes in growth hormone in the blood during day and night in normal subjects (1 16) It has become obvious that in some acromegalics too there may be large variations in plasma growth hormone from hour to hour (5 1) Furthermore, some acromegalic patients have a paradoxical rise in growth hormone following oral glucose (17) The high degree of variability of plasma growth hormone in these patients does suggest persistent hypothalamic control of growth hormone secretion (5 13).

The rapidly changing plasma levels of growth hormone and the fact that some acromegalic patients may have normal fasting plasma growth hormone values (3) make it of interest to study urinary growth hormone in these patients Glick (6) stated that there was no difference in urinary excretion of growth hormone in normal subjects and in acromegalics On the other hand Bala and Beck (1) Chakmakjian and Langston (4) and Hanssen (8) reported that some acromegalic patients had increased urinary growth hormone when compared to control subjects However they did not correlate urinary excretion to plasma growth hormone or to kidney function n these patient

The present paper describes urinary growth hormone in acromegalic patient compared to pla ma growth hormone during peroral glucose tolerance test in order to elucidate the relationship between plasma and urinary growth hormone in this cond tion. Furthermore the usefulness of measurements of urinary growth hormone as a diagnostic tool in acromegaly is demonstrated

SUBJECTS AND METHODS

W studied 16 patients with obvious clinical acromegaly They all had acral growth and all but one had increased fasting plasma growth hormone values. The clinical and laboratory data of the patients are show in Table I Two patients (no 1 and 7) ere studied before and after transfrontal hypophysectomy Five of the patients had been treated previously for acromegaly as show in Table I Twelve of the patients had an enlarged sella turcica on plain X-ray but only one had suprasellar extension of the tumour None had sual defects.

It is difficult to assess the clinical activity of acromegaly W have made an attempt to describe the patient status in thi respect by clinical criteria (acral growth wetting, paresthesia, pathological glucose tolerance) (Table I) All patients had normal serum creatinine Endogenous creatinine clearance was deter mined in 11 of the patients and ranged from 67 1 to 149 ml/min/173 m² (normal range 70–140). Three patient (nos. _ 9 and 10) had traces of albuminuria with albustix Peroral glucose tolerance test (1 g glucose/kg b wt) was performed for 3–4 hours in all patients cept the one with insulin-treated diabetes. Plasma growth hormone was determined every 30 min during the glucose tolerance test

The control group consisted of 9 persons (5

Table I. *Clinical data, plasma and urinary growth hormone (GH) values in acromegalics*

Pat. no.	Sex	Age (y.)	Fasting GH (ng/ml)	Mean plasma GH (ng/ml)	1-hour GH (% of 0 h)	Urinary GH (ng/24 h)	Glucose tolerance	Activity	Treatment
1	♂	28	79?	695	85	3 456	Borderline	+	None
			89	82	91	456	Normal		Transfrontal hypophysectomy
	♂	65	21	25.7	162	661	Normal	+	None
3	♂	64	30	?0.9	68	169	Chemical diabetes	+	None
4	♀	63	5.9	8.8	339	110	Normal	+	None
5	♀	57	47	49.6	117	113	Normal	+	X-ray 196.
6	♀	47	155	117.1	91	645	Chemical diabetes	+	None
7	♀	26	19.9			1 .38	Insulin-dependent	+	None
			9.9			129	Insulin dependent		Transfrontal hypophysectomy
8	♂	47	1..9	16.3	152	131	Borderline	–	None
9	♂	4?	57	51.7	91	156	Sulphonyl-rea	+	Transfrontal hypophysectomy 1966
10	♀	74	4	32.6	103	276	Borderline	–	X-ray 1951
11	♂	55	8.7	13.4	187	17	Normal	–	None
1	♀	69	1..8	?9.0	.36	166	Chemical diabetes		None
13	♀	70	14.1	31.4	226	363	Chemical diabetes	–	None
14	♀	59	5.6	5.8	98	340	Chemical diabetes	+	None
15	♂	46	13..	13.9	105	178	Normal	–	None
16	♀	40	25.9	24.6	83	323	Normal	–	None

4 women) earlier described (9). Plasma growth hormone was determined by radioimmunoassay (7). Normal fasting values were in men 1.48±0.33 and in women 3.83±3.47 ng/ml (mean ±S.D.). Urinary growth hormone was determined according to Hanssen (8) (normal range 17–64 ng/24 h urine). Statistical valuation was per .. d with non-parametric tests, Wilcoxon rank test Spearman correlation test.

RESULTS

Fasting and mean plasma growth hormone values during the peroral glucose tolerance test are shown in Table I. Plasma growth hormone values at 1 hour during the peroral test as percentage of 0 hour value were calculated as suggested by Wright et al. (17) to assess the paradoxical rise in growth hormone during peroral glucose seen in some patients (Table I). The relationship between mean plasma growth hormone and urinary growth hormone is shown in Fig. 1 ($R=0.40$, $p=0.1$). We could find no significant correlation between the paradoxical rise in growth hormone during glucose tolerance test and urinary growth hormone. The urinary growth hormone in acromegaly compared to the control group is shown in Fig. .. It is evident that all patients with acromegaly had increased urinary growth hormone ($p<0.01$). In this material we found no significant correlation between the clinical activity of the disease and plasma or urinary growth hormone (Table I).

It is of interest to study patient 14 with normal fasting plasma growth hormone and grossly increased urinary growth hormone.

Case history

A 59-year-old woman 7 years earlier treated with I[131] because of toxic goitre. At that time the sella turcica was enlarged (18×10 mm) and she had clinical signs pointing to acromegaly. In the last year before the present investigation she had experienced growth of the tongue, coarse voice and acral growth, indicating activity in the acromegalic process. Results of plasma growth hormone determination during peroral glucose tolerance test, arginine test and insulin tolerance test in this patient are shown in Fig. 3.

DISCUSSION

This study shows that measurement of urinary growth hormone may be used as a diagnostic procedure in acromegaly. All patients with acro-

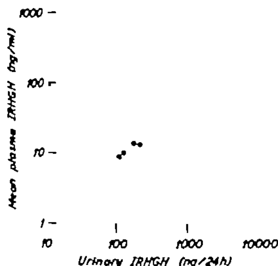


Fig. 1. Relationship between urinary and mean plasma growth hormone (*IRHGH*) during peroral glucose tolerance test in acromegalics.

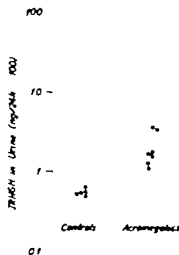


Fig. 2. Urinary growth hormone (*URHGH*) in controls and acromegalics.

megaly had increased urinary growth hormone when compared to a control group. This also applies to the patient with active acromegaly but without increased fasting growth hormone levels.

The mechanism behind the increased urinary growth hormone seen in acromegaly might theoretically be more than merely a reflection of the plasma growth hormone levels. It is well known that glomerular filtration rate may be elevated in acromegaly (15). The endogenous creatinine clearance was only slightly increased in one patient (no. 1) in this study. Thus an increased glomerular filtration rate cannot explain the considerably increased urinary growth hormone seen in acromegalics. It has been shown that patients with insulin-dependent diabetes mellitus have increased urinary growth hormone (9, 10). However, only one patient (no. 7) in the present study was treated with insulin and urinary growth hormone decreased 10 times in this patient following transfrontal hypophysectomy (Table I) although insulin treatment was continued.

Growth hormone is thought to be filtered by the glomerulus and reabsorbed for the most part in the proximal tubulus of the kidney (11–14). Thus inability of the proximal tubulus to reabsorb the growth hormone present in the ultrafiltrate may lead to increased urinary growth hormone. We are not aware of data of protein reabsorption in proximal renal tubulus in acromegaly, so it is not possible at the present time to say whether a proximal tubular dysfunction in acromegaly exists.

Previously other authors have been concerned with urinary growth hormone in acromegaly. Glick (6) studied urinary growth hormone in normal adults and acromegalics. He found no differences between these two groups. However, the meas-

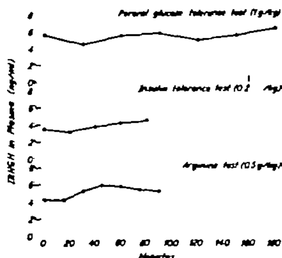


Fig. 3. Plasma growth hormone (*URHGH*) during peroral glucose tolerance test, insulin tolerance test and arginine test in patient 14.

urements were performed on unconcentrated urine and the normal values were about 10–70 times higher than our normal values Lowy et al (14) studied urinary growth hormone in renal failure and found that urinary growth hormone in acromegalics was lower than that in severe renal failure However they were not able to estimate the low values for urinary growth hormone found in normal subjects Bala and Beck (1) and Chakmakjian and Langston (4), in methodological studies on urinary growth hormone reported that some patients with acromegaly had increased urinary growth hormone but they did not compare with plasma growth hormone studies or renal function

The present study shows no significant correlation between mean plasma growth hormone during peroral glucose tolerance test and urinary growth hormone There may be several reasons for this As stated kidney function may influence the amount of growth hormone excreted in urine There is no evidence of serious kidney disease in this material. Thus it seems most reasonable to ascribe this rather moderate association between mean plasma and urinary growth hormone to the rapidly changing plasma growth hormone seen in acromegalics. It is seen that patient 14 with fasting plasma growth hormone within the normal range but with no response to peroral glucose insulin tolerance test and arginine infusion, had grossly elevated urinary growth hormone

Thus in conclusion estimation of urinary *th hormone is a useful diagnostic tool in the* diagnosis of acromegaly especially in cases where fasting plasma growth hormone is within the normal range and at the beginning of the development of acromegaly before the disfiguring changes have taken place Moreover urinary growth hormone determination is of interest when assessing the effect of treatment on growth hormone dynamics in acromegaly

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THE PROGNOSIS FOR PATIENTS WITH COMPLETE HEART BLOCK TREATED WITH PERMANENT PACEMAKER

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Abstract. A follow-up study of the survival rate of 164 patients with complete heart block treated with permanent pacemaker showed 87% survival after one year 76 after two, and 50% after five years. These figures indicate an excess mortality per year in the first five years of 6–7% compared with population with the same age and sex distribution. Analysis of the survival curve shows that the excess mortality is caused by other coexisting diseases, such as renal failure, diabetes or cancer, valvular heart diseases or other types of cardiovascular affections, or heart failure.

In a retrospective study of the prognosis of patients with complete heart block (CHB) Johansson (4) found a significant difference in 1 year survival dedepending on the dominant disease or etiology of CHB, varying from 80% in patients with rheumatic heart disease to 29% in patients with CHB produced by digitalis.

The purpose of this report is to present the survival of patients with CHB treated with permanent pacemaker in relation to clinical findings at the start of the pacemaker treatment.

PATIENTS AND METHODS

The report is based on 164 patients with CHB who started permanent pacemaker treatment in 1962 70. They have been followed up to death, or to the time when they left for control in another hospital, or to the last control in Department B before June 1st 1973. Only patients ho have follow-up period of at least two years have been included.

The indications, techniques, complications and follow-up procedures used in permanent pacemaker treatment in the Department are presented in two other reports (3 5). Survival calculations were performed as described by Bedford and Card (1). Calculation of the survival curves for a matched population was performed as described by Olesen (6), using the mortality table (Table 26) from "Statistiske Meddelelser" no. 7 1972.

Patients with intermittent and permanent CHB have been treated as one group, as Johansson (4) found no difference in survival in these two groups of patients. The patients were divided into 8 groups (Table I) based on the clinical findings t the start of pacemaker treatment.

Group I: 65 patients ithout coexisting disease and without heart failure. *Group II*: 17 patients in heart failure but without coexisting disease. *Group III*: 22 patients without heart failure but with coexisting diseases as seen from Table II. Ten had two or more coexisting diseases and 18 had cardiovascular diseases, renal failure and/or diabetes (nos. 1 7 in Table II). *Group IV*: 29 patients in heart failure and with coexisting diseases as seen from Table II. Eleven had two or more coexisting diseases and 28 had cardiovascular diseases, renal failure and/or diabetes (nos. 1 7 in Table II). *Group V*: 12 patients with valvular heart diseases. Nine had aortic valv affection (3 aortic stenosis, 2 aortic insufficiency 4 aortic stenosis and insufficiency), five ere in heart failure. Three patients had combined mitral stenosis and insufficiency tw were in heart failure. *Group VI*: four patients ith CHB after operation for atrial septal defect (2 patients) or for corrected transposition (2 patients). *Group VII*: eight patients with cancer verified before start of pacemaker treatment, with the exception of one in whom the pacemaker was implanted in connection with gastrectomy T patients had c. mammae, two c. pulmonis and one ventriculi, c. oeso-

Table I. *Age and sex distribution of the 164 patients studied*

Group	Males		Females		Total
		Mean age and range or age (y.)		Mean age and range or age (y.)	
I	42	70.5 (41–90)	23	71.0 (48–84)	65
II	12	75.1 (61–85)	5	74.1 (55–87)	17
III	17	73.0 (61–82)	5	63.1 (51 72)	22
IV	21	73.8 (52–87)	8	75.8 (68–84)	29
V	8	61.8 (42–76)	4	53 58 67 79	12
VI	2	27 29	2	7 17	4
VII	3	71 72 80	5	72.0 (55–81)	8
VIII	3	40 41 87	4	26 ?8 42 51	7
Total	108	70.4 (27–90)	56	66.5 (7–87)	164

Table II. *Coexisting diseases in 82 of the patients studied*

	Group III	IV	V–VIII	Total
1 Recent myocardial infarction	4	3		7
2 Previous myocardial infarction	4	6	1	11
3 Hypertension (diastolic pressure 100 mmHg)	3	4		7
4 Stroke	2	2		4
5 Intermittent claudication	2	4	2	8
6 Diabetes	5	11		16
7 Chronic renal failure	5	8		13
8 Polyarthritis	4		1	5
9 Chronic bronchitis	3	1	2	6
10 Gastroduodenal ulcer	1	1		2
11 Epilepsy	1		1	2
12 Myxedema	1			1
13 Gout		1		1
14 Valvular heart disease			12	12
15 Cancer			8	8
16 Congenital heart disease			5	5
17 Myocarditis/cardiomyopathy			3	3
18 Cirrhosis of the liver			1	1
19 Amaurosis			2	2
20 Sarcoidosis			1	1
21 Polymyositis			1	1
Total	22	29	31	82

phagi, c. recti and c. coli, respectively. One patient was in heart failure. *Group VIII*, seven patients with miscellaneous diseases. None of these patients were in heart failure.

Table III. *Observation time for the 164 patients studied*

Interval after start of pacemaker treatment (y)	Status at end of observation period: Survivals	Deaths	Total no. surviving at beginning of year
0–1	—	22	164
1–2	—	17	142
2–3	21	10	125
3–4	21	9	94
4–5	19	10	64
5–6	6	1	35
6–7	7	—	28
7–8	9	—	21
8–9	5	1	12
9–10	2	—	6
10–11	2	—	4
11 12	2	—	2
Total	94	70	164

RESULTS

Of 164 patients who started pacemaker treatment because of CHB, 142 (86.6%) were alive after one year and 125 (76.2%) after two years. After five years 50% are calculated to survive. No patients died in the sixth to eighth year The mortality per year is 10% for the first five years compared to 3 5% in a population matched for sex and age (Fig. 1 and Table III). Patients without heart failure and without coexisting disease (group I) have a calculated survival curve which is the same as that of a matched population (Fig. 2). After one, respectively two years 62 and 60 of the 65 patients were alive. The higher mean age in patients without coexisting diseases but in heart failure (group II) compared to patients without heart failure (group I) probably accounts for the higher mortality as the calculated survival curve is not different from the survival curve of a matched population (Fig. 3). Of the 17 patients, 16 were alive after one year and 13 after two years of observation.

Patients with cardiovascular diseases, renal diseases, diabetes and rheumatoid arthritis besides CHB but without heart failure (group III) have a calculated 5-year survival of 47% compared to 77 in a matched population (Fig. 4). Of 22 patients, 18 were alive after one year and 16 after two years.

Patients with the same diseases as in the previous group but with heart failure (group IV) have a significantly higher mortality than both the matched population and the patients without heart failure. The calculated survival curves for the populations matched to groups III and IV are identical, showing 76% alive after five years. Of 29 patients in group IV 22 were alive after one year and 16 after two years, and 20% are calculated to live after five years (Fig. 5).

All patients with valvular heart disease (group V) died in the observation period, three in the first and second year one in the third year two in the fourth and fifth year and one in the eighth year One of the patients died postoperatively after insertion of aortic valve prosthesis after pacemaker treatment for 56 months. After one, respectively two years 75 and 50% of the patients were alive. One patient was alive after five years. This represents an excess mortality of about 80% in comparison with a matched population.

Two patients developed CHB after operation for atrial septal defect at the ages 7 respectively 29 years. Both patients were alive at the end of the observation

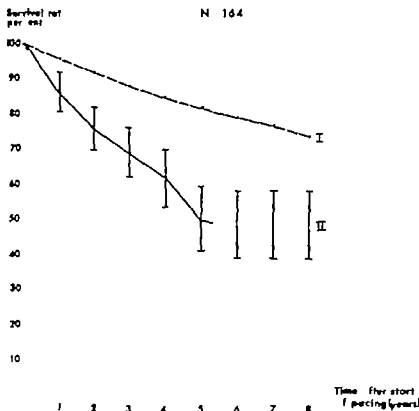


Fig. 1. Survival curve of 164 patients with CHB treated with permanent pacemaker (*II*) and the calculated survival curve of a population with the same age and sex distribution (*I*). Vertical bars indicate ±2 S.E.M.

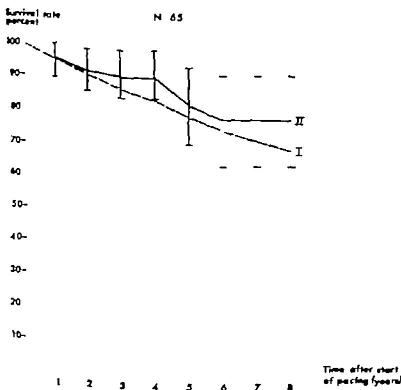


Fig. 2. Survival curve of group I (*II*) and the calculated survival curve of a population with the same age and sex distribution (*I*). Vertical bars indicate ±2 S.E.M.

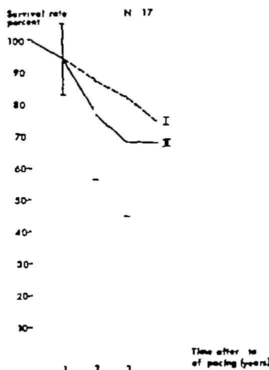


Fig 3 Survival curv of group II (*II*) and the calculated survival curve of population with the same ge and sex distribution (*I*). Vertical bars indicate ±2 S.E.M.



Fig 4 Survival curve of group III (*II*) and the calculated survival curve of a population with the same age and sex distribution (*I*). Vertical bars indicate ±2 S.E.M.

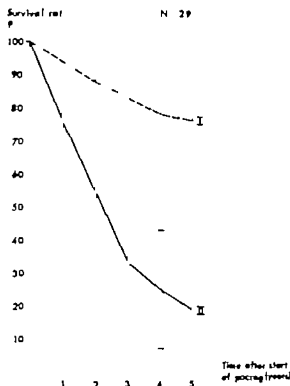


Fig 5 Survival curve of group IV (*II*) and the calculated survival curve of population with the same age and sex distribution (*I*). Vertical bars indicate ±2 S E.M.

period after 77 and 111 months pacing. Two patients with corrected transposition and tetralogy of Fallot developed CHB after radical operation a 17-year old female was alive at the end of the observation period after 47 months' pacing a 27-year-old male died from sepsis and heart failure after 2 months' pacing.

Eight patients with cancer known when the pacing was started (group VII) died during the observation period. One died postoperatively after insertion of epicardial electrodes, and two others in the first, one in the second, two in the third, and two in the fourth year This means that 50 % were alive after two years and none after four years.

In group VIII one patient, an 87 year-old male with hyperexcitable carotic sinus, died in the second year a 40-year-old male with polymyositis in the fourth, and a 51 year-old female with sarcoidosis in the fifth year Four patients were alive at the end of the observation period after 42, 47 74 and 134 months treatment, respectively with permanent pacemaker All were alive after one year and 86% after two and three years, which means that the sur

vival curves for these patients and for the patients in groups I and II are not different.

DISCUSSION

It is generally agreed that the outlook for patients with CHB has improved markedly after the introduction of permanent pacemaker therapy. The survival curve of our patients (Fig. 1) is in agreement with other pacemaker-treated series (2, 7, 8).

Excluding patients with CHB produced by acute myocardial infarction and digitalis, Johansson (4) found a 1-year survival of 60%. The 1 year survival was dependent on the dominant disease or the etiology of CHB. It was found to be 80% in rheumatic heart disease, 63% in CHB of unknown etiology, 56% in hypertension and 43% in patients with no acute coronary heart disease. These figures illustrate the importance of the etiology and of coexisting diseases for the prognoses of patients with CHB.

The 1-year survival of 87% in our patients is significantly better than the 60% in Johansson's patients. Nevertheless an excess mortality per year of 6-7% is seen in the first five years in the patients with CHB treated with permanent pacemaker compared with a population matched for sex and age.

Analysis of the survival curve (Fig. 1) shows that the excess mortality is caused by: 1. patients with coexisting diseases (primarily cardiovascular and renal diseases and diabetes), 2. patients in heart failure, 3. patients with valvular heart disease, and 4. patients with cancer.

It may be concluded that after the introduction of permanent pacing the prognosis for patients with complete heart block is determined by the other diseases from which the patient may suffer and not by the block itself.

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ACUTE MYOCARDIAL INFARCTION COMPLICATED BY THIRD DEGREE ATRIOVENTRICULAR BLOCK TREATED WITH TEMPORARY PACEMAKER

Hospital and Long-term Survival in 57 Patients

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Abstract. During the period Jan. 1 1968 – Dec. 31 1970, 57 patients with acute myocardial infarction (AMI) complicated by third degree atrioventricular (AV) block have been treated with temporary pacing. The hospital lethality in anterior AMI (75%) was significantly higher than in inferior AMI (22%). Cardiogenic shock, Adams–Stokes attacks, prolonged QRS complex and development of third degree AV block directly from sinus rhythm occurred significantly more frequently in anterior wall infarction and were followed by a significantly higher lethality. Age, previous AMI, ventricular fibrillation, route of admission, degree of cardiac incompensation, number of Adams–Stokes attacks or asystole, heart rate prior to pacing and time from onset of AMI to third degree AV block were found to be of no importance for the lethality. The cumulative survival rate was 50% at 12 and 47% at 24 months after the infarction. In anterior AMI it was 15% at 12 months and in inferior AMI 73% and 65% at 12 and 24 months, respectively. The long-term prognosis was not improved in patients with anterior AMI treated by pacemaker compared to medically treated patients. In inferior AMI the importance of pacemaker treatment is not yet clarified. Based on the literature and our own results it is concluded that patients with AMI complicated by third degree AV block should still be treated with pacemaker.

Acute myocardial infarction (AMI) is complicated by third degree atrioventricular (AV) block in 5–12% of the cases (2, 5) and was previously followed by a lethality of 50–77% on medical treatment (4, 5). During recent years pacemaker treatment has been universally employed and a convincing improvement in survival rate was expected. However most investigations have reported lethalities of 28–50% during pacemaker treatment (9 13 15 16, 17), which are of the same magnitude as found by Christiansen et al. (3) and Wole and Aksnes (18), who reported lethalities of 38 and 53%, respectively when AMI complicated by third degree AV block was treated medically.

The purpose of this report is, based on the clinical condition at the initiation of pacemaker treatment, to describe factors which may influence the early and late prognosis in patients with AMI complicated by third degree AV block and to discuss the indications for temporary pacemaker treatment.

PATIENTS AND METHODS

This study comprises all patients admitted to Medical Department B, Rigshospitalet, during the period Jan. 1 1968 – Dec. 31 1970, with AMI complicated by third degree AV block treated with pacemaker.

The diagnosis was considered established either when the infarction was demonstrated at autopsy or when two of the following three criteria were fulfilled: 1) typical ECG findings with progressive development of ST elevation, negative T and Q waves, 2) a typical clinical course with retrosternal pains, 3) an abnormal increase in serum LDH.

One patient, who had LBBB prior to the third degree AV block, will be mentioned separately. One patient, who had anterior as well as inferior wall infarction at autopsy is classified as an anterior wall infarction.

Forty-three patients were males of mean age 61.8 years (range 37–86) and 13 females of mean age 68.8 years (range 56–76). Twenty-nine patients were referred directly to the Medical Department either by general practitioners or via the Emergency Ward, whereas 27 were transferred from other hospitals in Copenhagen or from county hospitals up to a distance of 150 km from Copenhagen. No significant difference in sex, age or clinical condition was found among these groups of patients, and they are consequently regarded as one group. Sex and age distribution and infarct localisation are shown in Table I.

Cardiac arrest or severe tachyarrhythmias have been treated according to Hansen and Sandøe (6). In temporary pacemaker treatment an Elema electrode (type EMT 588) was implanted through an arm vein and placed in the apex of the right ventricle according to the technique described by

Table I *Sex and age distribution and site of infarction in 56 patients with AMI complicated by third degree AV block*

	Site of infarction	Age (yr) <55	55–64	65–74	>75	Total
Males	Anterior	5	5	6	2	18
	Inferior	5	11	8	1	25
Females	Anterior	0	0	2	0	2
	Inferior	0	2	7	2	11
Total		10	18	23	5	56

Melbom et al. (12). In emergency cases or in cases when it was found difficult to place an Elema electrode, bipolar catheter (USCI no. 5161) was used. The electrode was connected to n external pacemaker (Medtronic type 5840 or 5880). Pacing on demand with rate 60–80/min was used in all cases.

Statistical evaluation of the findings was done by standard procedures (χ^2-test or Fischer test). P-values below 0.05 were considered significant.

Information concerning the survival of the patients was obtained from the Folkeregisteret in May 1972. This means that all patients were followed for t least 16 months except for one patient who emigrated after two months.

The cumulative survival rate was calculated according to Bedford and Caird (1).

RESULTS

Anterior and inferior AMI did not differ significant relation to sex and age. A significantly larger of patients with Adams–Stokes attacks, duration >0.12 sec, and shock were found in nterior AMI, whereas a significantly higher incidence of first and/or second degree AV block prior to start of pacemaker treatment was found in posterior AMI (Table II). There was no significant difference between anterior and inferior wall infarction in relation to the following factors route of admission (directly or through other hospitals) cardiac incompensation, asystole, ventricular fibrillation, previous myocardial infarction, or the heart rate prior to pacemaker treatment.

Thirty patients developed third degree AV block during the first 24 hours, in 19 patients it was observed on the second to fourth day and in 6 it occurred after the fourth day. In one patient the time of occurrence of third degree AV block was unknown.

Of 23 patients with third degree AV block who died in the department, 9 developed sinus rhythm whereas 14 had third degree AV block at death. Of 33 patients discharged from hospital 3 received a permanent pacemaker (one had intermittent and two permanent third degree AV block, one of the latter had had the block for 7 years before the infarction). Among the remaining 30 patients 11 had third degree AV block for less than one day 5 for 1–3 days, 8 for 4–6, 3 for 7–10 and 3 for more than 10 days (12, 13 and 19 days). Twenty-nine of these patients had sinus rhythm and one patient had atrial fibrillation which was also present before the infarction.

One patient (female, 69 years) was transferred from another hospital with third degree AV block which occurred after the AMI. Localization of the infarction was not possible as the patient had had LBBB before admission. The patient had had asystole and Adams–Stokes seizures but no cerebral damage. A temporary pacemaker electrode was inserted and kept in place for 21 days but was only used during the first two days. The patient was discharged with sinus rhythm after 37 days of hospitalization and died nine months later. The cause of death is unknown.

Table II *Site of infarction in relation to clinical and ECG findings at start of pacemaker treatment in 56 patients with AMI complicated by third degree AV block*

Clinical and ECG findings prior to pacemaker treatment		Site of infarction: Anterior	Inferior	p
Adams–Stokes syndrome	+	14	15	<0.05
	−	6	21	
Asystole	+	10	12	>0.5
	−	10	24	
Ventricular fibrillation	+	5	3	>0.2
	−	15	33	
Duration of QRS complex <0.12 sec	+	4	22	<0.02
	−	16	14	
Shock	+	11	9	<0.05
	−	9	27	
Previous myocardial infarc.	+	5	8	>0.2
	−	15	28	
1st and 2nd degree AV block*	+	3	15	<0.05
	−	14	14	

* 1 10 patients the first ECG recording showed third degree AV block (3 anterior and 7 inferior infarctions).

Table III. *Clinical and ECG findings in relation to lethality in 56 patients with AMI complicated by third degree AV block*

	Site of infarction								
	Anterior		Inferior		Total				
	No. of pats.	No. of deaths	No. of pats.	No. of deaths	No. of pats.	No. of deaths	Lethality ( )	p	
Age (y)									
65	10	6	18	3	28	9	32	>0.3	
>65	10	9	18	5	28	14	50		
Normotensive	4	1	19	1	23	2	9	>0	<0.001
Hypotensive	5	3	8	1	13	4	31	<0.01	
Shock	11	11	9	6	20	17	85		
Duration of QRS complex (sec)									
>0.12	16	13	14	7	30	20	67	<0.001	
0.12	4	2	22	1	26	3	12		
Previous myocardial infarction									
+	5	4	8	4	13	8	62	<0.2	
–	15	11	28	4	43	15	35		
Ventricular fibrillation prior to 3rd degree AV block									
	5	4	3	1	8	5	63	<0.1	
	15	11	33	7	48	18	38		
1st and 2nd degree AV block prior to 3rd degree AV block									
	3	0	15	1	18	1	6	<0.001	
–	14	12	1	4	28	16	57		

Hospital lethality

The hospital lethality in patients with AMI and third degree AV block was 41%. The lethality in anterior AMI (75%) was significantly higher than in inferior AMI (22%) ($p < 0.01$). Table III shows the factors considered of importance for the hospital lethality in relation to the site of the infarction.

Age. The most pronounced difference in lethality was found when comparing patients below 65 (32%) and above 65 years (50%), but the difference was not significant ($p > 0.2$).

Blood pressure and shock. Twenty-three patients had normal BP (systolic >100 mmHg), 13 had hypotension and 20 were in cardiogenic shock. The lethality for these three groups of patients was 9 31 and 85%, respectively. The differences in lethality between patients with normal BP and patients with hypotension was not significant ($p > 0.2$). When normotensive and hypotensive patients were compared to patients in cardiogenic shock, the difference in lethality was found to be significant ($p < 0.001$ and p 0.01 respectively). There was no significant difference between the lethality in inferior and anterior AMI for the three groups. However it should be stressed that the lethality in anterior AMI complicated by cardiogenic shock was 100 %.

Duration of QRS. A significantly higher lethality ($p < 0.001$) was found when the QRS complex duration was >0.12 sec, irrespective of the site of the infarction. One of 22 patients with inferior AMI and QRS <0.12 sec died. This patient was admitted after a prolonged cardiac arrest. The patient developed sinus rhythm a few hours after the development of the block but died on the second day unconscious in uraemia.

Previous AMI was found in 13 patients but was not followed by a significantly higher lethality ($p > 0.1$).

Ventricular fibrillation occurred in 8 patients prior to the development of the third degree AV block. The lethality for these patients was 63 %, which was not significantly higher ($p > 0.05$) than that of patients in whom ventricular fibrillation was not observed.

First and/or second degree AV block prior to third degree AV block. Of 3 patients with anterior and 15 with inferior AMI in whom first and, or second degree AV block occurred prior to third degree AV block, one died (6%). Among 14 patients with anterior and

14 with inferior AMI, who changed to third degree AV block directly from sinus rhythm, 12 and 4 died, respectively. The total lethality in this group was 57%, which was significantly higher ($p < 0.001$) than in patients with third degree AV block preceded by first or second degree AV block.

Other factors. No significant differences in lethality were observed in relation to the following factors: route of admission, degree of cardiac incompensation, number of Adams–Stokes attacks or asystole, heart rate prior to the implantation, or the duration from the first signs of AMI to the development of third degree AV block.

Long-term survival

The total cumulative survival rate in 56 patients was 50% (2 S.E. 36–63%) at 12 months and 47% (2 S.E. 3–61%) at 24 months after the infarction. In 33 patients discharged from the hospital the observation period ranged from 1 to 45 months, during which a total of 7 patients died.

The total cumulative survival rate for 20 patients with anterior AMI was 15% (2 S.E. 1–31%) after 12 months of observation. Among 5 patients with anterior AMI two died 1 and 7 weeks after the discharge, respectively, whereas three were alive at the end of the observation period. The mean observation period for these 5 patients was 17 months (range 1–43) and the total observation time 84 months, corresponding to one death per 42 months. The total cumulative survival rate for 36 patients with inferior AMI was 73% (2 S.E. 57–87%) at 12 months and 65% (2 S.E. 49–81%) at 24 months after the infarction. Among 28 patients with inferior AMI one has not been traced (emigrant), five died after 2, 4, 7, 13 and 34 months, respectively, whereas the remaining 22 patients were alive at the end of the observation period. For 27 patients discharged alive the mean observation time was 21 months (range 2–45) and the total observation time 577 months, corresponding to one death per 115 months.

None of the factors analysed in relation to the hospital lethality were found to be of importance for the long-term survival.

DISCUSSION

The hospital lethality for patients with anterior AMI complicated by third degree AV block in this study was 75%. This lethality is in accordance with medically as well as pacemaker-treated series (3, 7, 8, 10), except for that of Wole and Aksnes (18), who found a strikingly low lethality (33.3%), probably due to an unusually high precentage of infarctions of uncertain localization (8 of 25 patients).

In our study the hospital lethality in inferior AMI was 22%, which is significantly lower than the 50% found by Johansson (7) and Wole and Aksnes (18). Christiansen et al. (3) found a lethality of 19% in their patients.

The overall mortality is very difficult to compare in different series of pacemaker-treated patients because of differences in selection of patients and in age, localization and extension of the infarction. The same difficulties arise when comparing with medically treated series and, furthermore, these often include patients who have been treated with temporary pacemaker. For these reasons we have analysed the various factors which might influence the hospital lethality.

In this series it was found that shock, broadened QRS complex and the development of third degree AV block directly from sinus rhythm indicate a significantly higher hospital lethality. The higher hospital lethality in anterior AMI compared to inferior AMI is explained by the higher incidence of these factors, which has also been found by others (3, 11). The anatomical explanation of these findings is reviewed in detail by Resnekof and Lipp (14).

Our material has further been analysed for the prognostic significance of the following factors: route of admission, age, previous myocardial infarction, ventricular fibrillation, heart failure, Adams–Stokes attacks, asystole, heart rate prior to pacemaker treatment and time for the development of third degree AV block after the onset of AMI. None of these factors were found to be of significance for the hospital lethality. These findings are in accordance with those of others, except for Scott et al. (16) who found that hospital lethality increased significantly when the block developed within 24 hours of infarction if the patients were over 65 years of age or had had Adams–Stokes attacks.

Investigations concerning long-term survival in medically treated patients with AMI complicated by third degree AV block comparable to the present series are not available.

In inferior AMI the 1 year survival in our patients was 73%, which corresponds well to a survival of 69% found by Julian et al. (8). In inferior AMI with QRS < 0.12 sec the 1 year survival rate of 85%

is of the same magnitude as for large series of medically treated patients in coronary care units. In anterior AMI the prognosis was poor as only 15% of the patients were alive after 12 months and all patients with broadened QRS and shock died within 2 months. This is the same as described by Julian et al. (8) and Johansson (7) and substantiates the fact that in anterior AMI complicated by third degree AV block the damage to the myocardium is often extensive and determines the poor prognosis irrespective of the treatment, whereas in inferior AMI complicated by third degree AV block the myocardial lesion is often small and explains the better prognosis (3, 10).

It has not yet been clarified whether acute pacemaker treatment is indicated in all patients with AMI complicated by third degree AV block. It is well known that a dramatic improvement in the clinical and haemodynamic condition can be seen when pacemaker treatment is employed (17). Furthermore the high incidence of Adams–Stokes attacks seen in AMI complicated by third degree AV block, irrespective of the site of the infarction, implies a high risk for these patients, which is in accordance with Johansson (7), who found that at least 13 of 34 patients died from Adams–Stokes attacks. For these reasons we shall continue to treat all patients with AMI complicated by third degree AV block with pacemaker although a significant improvement in survival could not be demonstrated.

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THE EFFECT OF PROPRANOLOL ON PLASMA RENIN ACTIVITY AND BLOOD PRESSURE IN MILD ESSENTIAL HYPERTENSION

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Abstract. Fourteen male patients with mild to moderate essential hypertension have been studied with regard to plasma renin activity (PRA) after acute and prolonged β-adrenergic blockade with propranolol. Initial PRA was determined after four weeks of placebo treatment. Before propranolol was given PRA rose in response to 10 min of 45° head-up tilt from 151.7 ng/100 ml/h to 248.7 ng/100 ml/h ($p<0.01$). After acute administration of propranolol 0.22 mg/kg b. i. d. and following repeated tilt for 10 min PRA only rose to 204.7 ng/100 ml/h (n. s.). Following four weeks of oral propranolol treatment at 160–320 mg daily PRA after tilt was 39.0 ng/100 ml/h. Thus a significant reduction of PRA had taken place ($p<0.005$) to a level constituting only 15% of the initial PRA after tilt.

The reduction of blood pressure (BP) after four weeks of propranolol treatment was also significant. Diastolic BP was reduced by 19 mmHg ($p<0.001$). The changes in BP and tilted PRA were not significantly correlated ($r=0.449$, $p<0.10$). These results indicate that propranolol causes a marked reduction of PRA in addition to its hypotensive effect. However, this does not necessarily imply that there is a direct causal relationship between the effect of propranolol on PRA and its effect on BP.

Usually renin is not elevated in patients with non-malignant essential hypertension (7, 12, 29) and aldosterone secretion is normal (7, 19). On the other hand, elevated renin levels and increased aldosterone secretion practically always occur in malignant hypertension (16, 17, 20). Already in 1960 Laragh et al. suggested that the vascular damage seen in malignant hypertension was related to elevated levels of angiotensin (16, 17). Later animal studies have shown that excess renin/angiotensin may cause vascular lesions of the same appearance as those seen in malignant hypertension (5, 8, 9, 22).

Recently Laragh's group presented observations on 219 hypertensive patients followed for 10 years, which indicated that low plasma renin was associated with a reduced risk of cardiovascular complications such as stroke and myocardial infarction (2). Others do not support this view (6).

Based on observations by Laragh and coworkers (2), it would be logical to use antihypertensive therapy that not only reduces blood pressure (BP) but also lowers renin actively. Several of the antihypertensive drugs have been studied with regard to their effect on plasma renin activity (PRA).

Thus diuretics (1, 28), hydralazine (21), diazoxide (15) and sodium nitroprusside (14) have been shown to cause elevations of PRA, while drugs with adrenergic inhibitory effects may cause mild to moderate reductions of PRA. This has been demonstrated e.g. for methyldopa (23), clonidine (13, 24), phentolamine (30), propranolol (30) and alprenolol (4).

As propranolol, at least in acute experiments, seems to be quite effective in reducing PRA (3) and as the experience with propranolol in hypertension has been encouraging (11, 26, 31) the present study was designed to study the effect on PRA of acute and chronic β-adrenergic blockade with propranolol in patients with essential hypertension.

MATERIAL

Fourteen male patients, 11 Caucasian, 3 Black, with mild to moderate essential hypertension were studied. Their average age was 45 years (range 27–46). Further details on the patients are recorded in Table I. Secondary causes for hypertension were excluded (physical examination, rapid sequence urograms or renal arteriograms, serum electrolytes and urinary catecholamines and aldosterone). Finally patients with history of bronchial asthma or cardiac failure were not included in the study.

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Table I. *Patient information*

Pat. no.	Age (y.)	Race[a]	Eye grounds[b]	Serum creatinine (mg/100 ml)	LVH[c]	Initial BP[d] (mmHg)
1	50	C	I	0.9	–	159/98
2	50	B	II	1.1	–	150/110
3	55	B	I	1.0	–	135/110
4	53	C	I	1.0	–	180/120
5	66	C	I	1.2	–	169/86
6	40	C	II	1.6	–	174/137
7	27	B	0	1.1	–	134/95
8	52	C	I	0.9	–	155/108
9	49	C	II	1.1	+	200/128
10	45	C	II	1.2	–	161/111
11	44	C	I	1.0	–	177/107
12	41	C	II	1.1	+	184/105
13	43	C	II	1.0	–	140/96
14	35	C	II	0.9	–	162/110

[a] C = Caucasian, B = Black.
[b] Keith, Wagener and Barker classification.
[c] Left ventricular hypertrophy in ECG or chest X-ray.
[d] Measured after 15 min recumbency in the clinic following 4 weeks of placebo treatment.

METHODS

After informed consent was obtained, all patients were given placebo as the only treatment for four weeks. They were then admitted to the Clinical Research Unit for four days. During these days baseline laboratory studies were performed. A no-added-salt diet (<100 mEq Na daily) was introduced in order to avoid excessive salt intake and the patients were instructed daily by a dietician regarding the continuation of this diet following discharge from the hospital.

On the morning of the fourth day the initial samples for PRA determinations were drawn. A catheter was placed in the left brachial artery with the patient in the resting recumbent position. After approximately 45 min of rest the first blood sample was drawn. The patients were then tilted to a 45° head-up position for 10 min and a second blood sample was drawn.

After recumbency had been resumed for a period of 45 min, propranolol was administered 0.22 mg/kg b.wt. Following a repeated 10-min period of tilt a third blood sample was drawn.

The patients were then discharged with propranolol orally 40 mg four times daily. They were advised to continue the no-added-salt diet. After two weeks propranolol was either increased to 80 mg four times daily or kept unchanged (if a diastolic BP reduction of 15 mmHg or more was recorded). The average daily dosage of propranolol during weeks three and four was 250 mg.

The fourth sample for PRA determination was collected after four weeks of oral propranolol treatment using the same set-up and under identical conditions as during the initial sampling. The fourth arterial sample was drawn after 10 min of tilt.

Blood pressure measurements

Office BPs were recorded by the same two nurses throughout the study. Their correlation was checked frequently by simultaneous readings using a "Y" connection. Readings were always at the same time of day in the recumbent position after 15 min rest in a quiet air-conditioned room. Phase V (disappearance) of Korotkoff sounds was taken as the diastolic endpoint.

Analysis of plasma renin activity

For each sample 10 ml arterial blood was collected in a prechilled tube containing approximately 10 mg EDTA-Na. The tube was immediately placed in ice and centrifuged within 20 min in a refrigerated centrifuge to recover the plasma. The plasma samples were then frozen and stored until all samples were collected. For analysis a radioimmunoassay technique was used (10, 27) by which PRA is determined by measuring the generated angiotensin I (Schwarz/Mann, Orangeburg, N.Y.). Duplicate analyses were made of all specimens and the results were compared to a standard curve obtained from standard solutions of angiotensin I. Nonspecific activity was determined in specimen no. 1 and subtracted from specimen nos. 1–3 and again determined and subtracted from specimen no. 4. The error of duplicate determinations varied between 4.2% and 7.9%.

Urinary sodium excretion

A 24-hour urine collection, ending on the morning of the fourth day was made for determination of sodium excretion.

Assessment of β-adrenergic blockade

Infusion of i.v. isoproterenol, 3 μg/min for 3 min, was used for estimation of the degree of β-adrenergic blockade. The change of heart rate was calculated from a continuous ECG tracing. This test was performed in all patients in the initial untreated state and was repeated after acute i.v. as well as after chronic oral administration of propranolol.

RESULTS

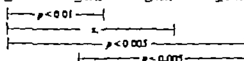
Table II presents the individual results regarding PRA in the initial untreated state and after acute and chronic β-adrenergic blockade with propranolol as well as sodium excretion and change of diastolic pressure. The average resting PRA was 151.7 ng/100 ml/h and rose to 248.7 ng/100 ml/h after 10 min of tilt ($p < 0.01$). Renewed tilt after i.v. propranolol produced a smaller increase of PRA to 204.7 ng/100 ml/h (n.s.).

After four weeks of oral propranolol, PRA after 10 min of tilt was 39.0 ng/100 ml/h. This is significantly lower than both the initial recumbent PRA ($p < 0.005$) and the initial tilted PRA ($p < 0.005$) (Fig. 1). Expressed as % of the corresponding initial tilted PRA the level after four weeks of oral propranolol was reduced by 85%.

Table II. *Plasma renin activity (ng/100 ml/h), sodium excretion (mEq/24 h) and blood pressure reduction (mmHg)*

Pat. no.	Urinary sodium	PRA rest placebo	PRA tilt placebo	PRA tilt prop. i.v.[a]	PRA tilt prop. orally[b]	Δ Diastolic BP[c]
1	160	40.3	59.4	27.6	14.0	−33
2	67	228.2	434.0	318.0	89.2	−17
3	105	54.4	149.0	68.9	12.4	−3[illegible]
4	55	443.4	709.3	722.9	20.4	−29
5	46	48.8	52.7	N.A.	10.7	9
6	171	461.3	875.6	739.6	93.4	29
7	158	45.4	68.3	32.6	38.1	11
8	94	113.3	135.9	60.9	16.5	28
9	N.A.	191.9	146.4	93.0	6.9	15
10	N.A.	123.4	151.4	76.2	39.7	−12
11	58	51.7	82.4	49.7	7.9	14
12	101	76.5	148.4	142.5	90.1	−11
13	68	204.7	406.5	304.7	104.4	−14
14	93	37.9	62.2	24.9	1.7	12
Average	98.0	151.7	248.7	204.7	39.0	19
S.E.M.	±12.6	±38.2	±69.8	±70.1	±10.1	±[illegible]4

Significance:



[a] Propranolol 0.22 mg/kg.
[b] Propranolol for four weeks, 160–320 mg daily.
[c] Change after four weeks of oral propranolol treatment.
N.A. not available.

Recumbent resting BP also fell in all patients as can be seen in Table II. The average drop of diastolic BP was 19 mmHg ($p < 0.001$).

The change of PRA from the initial tilted position to tilt after four weeks of oral propranolol was not significantly correlated to the reduction of diastolic BP ($r = 0.449$, $p < 0.10$).

That clinically significant β-adrenergic blockade was obtained both after i.v. and oral propranolol is illustrated by the effect of isoproterenol infusions. During placebo treatment the heart rate rose from 73 to 112 beats/min ($p < 0.001$). After i.v. propranolol repeated infusion of isoproterenol increased heart rate from 66 to 67 beats/min and following four weeks of oral propranolol isoproterenol infusion increased the heart rate from 57 to 62 beats/min.

DISCUSSION

It has previously been demonstrated that acute administration of propranolol can prevent or reduce an expected rise of PRA in response to upright posture (30). On the other hand Castenfors et al. (6), using chronic administration of alprenolol together with chlorthalidone, found that although PRA was reduced both in the recumbent and erect position the orthostatic increase of PRA was not abolished. We cannot exclude the possibility of a small increase of PRA due to tilt after acute or prolonged β-blockade as recumbent PRA was not measured after propranolol, but clearly any such increase must have been minute.

The effects of chronic oral administration of propranolol are quite striking, causing a marked reduction of PRA. Thus, comparing PRA after tilt in the untreated state and after oral propranolol it can be seen that PRA after chronic β-adrenergic blockade is only 15% of the initial level. Absolute final values in individual patients were either low or exceptionally low. The poor correlation between reduction of PRA and reduction of BP may be due to the fact that, although PRA fell drastically in all patients, the BP change was variable within the group. For this reason we cannot support the hypothesis of Bühler et al. (3) that induced change in PRA is a primary factor underlying the antihypertensive effect of β-adrenergic blockade.

It should be pointed out, though, that a direct

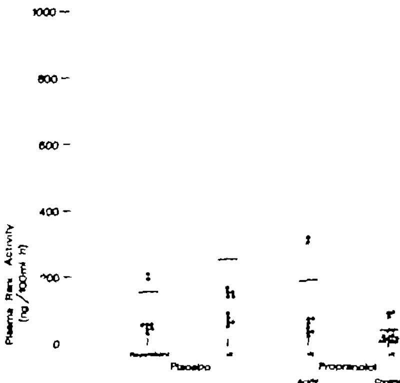


Fig. 1. PRA in 14 patients with essential hypertension. *Placebo* = measurements after 4 weeks of placebo treatment. *Recumbent* = horizontal rest for at least 45 min. *Tilt* = 45° head-up tilt for 10 min. *Acute Propranolol* = 0.22 mg/kg b.wt. i. *Chronic Propranolol* oral treatment for 4 weeks ith 160–320 mg daily.

PRA after tilt during placebo is significantly higher than in the recumbent position ($p<0.01$). PRA after chronic propranolol is significantly lower than after placebo both when compared to the recumbent value ($p<0.005$) and the tilted value ($p<0.005$) (paired *t*-test).

comparison with the results of Bühler et al. is not possible as different methods were used for analyzing PRA. Furthermore, our conclusions are based on stimulated (tilted) PRA and confined to patients with mild essential hypertension. Likewise, although low PRA hypertensives as a group did not respond as well to propranolol as those with normal PRA, variability of this phenomenon was such that found it to be statistically insignificant. Finally e dosage of propranolol was almost twice as high in the present study in comparison with the study of Bühler et al. Our interpretation of these results is tempered by the fact that the number of patients in this study was relatively small and that none had renovascular hypertension or a remarkably high initial PRA.

The rapid changes of PRA following change of posture that were observed in the present study confirm previous results by others (25). Finally sodium excretion was not studied at the end of four weeks of propranolol therapy. However as the patients were instructed by a dietician to maintain a "normal" diet and to avoid excess salt intake it is not logical to assume that the observed drastic changes of PRA were significantly influenced by possible minor variations of salt intake.

Regardless of its effect on BP it is clear that chronic administration of propranolol induces a low renin state. The clinical importance of this finding, i.e. its relation to the potential reduction of cardiovascular complications to hypertension (2), deserves further investigation.

ACKNOWLEDGEMENTS

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THE ACCURACY OF AUSCULTATORY MEASUREMENT OF ARM BLOOD PRESSURE IN VERY OBESE SUBJECTS

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Abstract. In 28 very obese subjects with arm circumference equal to or greater than 35 cm (mean 41.8, range 35–49) intraarterial measurements of arm blood pressure (BP) were performed simultaneously with auscultatory measurements using standard cuff (12 × 23 cm) and big cuff (14 × 45 cm). The mean difference between auscultatory and intraarterial measurements using the standard cuff was +6.8 mmHg (S.D. 14.3) for systolic and +14.3 mmHg (S.D. 9.7) for diastolic BP (phase V). Using the big cuff these mean differences were reduced to −5.8 mmHg (S.D. 11.2) and +7.1 mmHg (S.D. 7.1), respectively. Neither arm circumference nor BP correlated significantly with the difference between auscultatory and intraarterial measurement. It is emphasized that with the standard cuff the measurement of arm BP in these obese subjects is inaccurate. Using the big cuff the accuracy is better, similar to that of indirect measurements in normal subjects. But big interindividual differences nevertheless occur which cannot be predicted from the BP nor the circumference of the arm. Therefore, in patients in whom cuff hypertension is suspected, intraarterial measurements are indicated before prolonged antihypertensive treatment is initiated.

It has earlier been shown that indirect measurement of arm BP by auscultation is less accurate in subjects with very obese arms than in normal subjects (Table III). The tendency was that both the systolic and diastolic BP in these very obese subjects were 20–30 mmHg higher than the values from intraarterial measurements, but big interindividual differences were encountered. In normal subjects it is recommended that the length of the bladder should be equal to or longer than the circumference of the arm, and the width equal to the diameter of the extremity + 20% (7, 1). In the studies of very obese subjects the dimension of the cuffs did not fulfil these ordinary criteria of length and width.

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The purpose of the present study was therefore to compare indirect and direct BP measurements using both a standard cuff and a cuff which fulfilled the above mentioned criteria.

MATERIAL AND METHOD

Two groups of patients were studied. The first group consisted of 28 very obese subjects (19 females, 9 males) aged 18–69 years (mean 42). In all these subjects the circumference of the arm equalled or exceeded 35 cm (mean 41.8, range 35–49). The mean body weight was 122 kg (range 93–148).

The second group consisted of 21 subjects (6 females, 15 males) with arm circumference 22–34 cm (mean 28.3). Their mean age was 52.6 years (range 24–70) and mean weight 74.4 kg (range 51–100). These subjects were randomly selected among patients from an out-patient clinic in order to get subjects with normal arm dimension but with the same variation of systemic BP as for the obese subjects.

Indirect measurements

The subjects were allowed to rest in supine position for 20 min, and indirect measurement of arm BP was performed on the right and the left arm in order to compare the two sides. The pressures were equal within ±5 mmHg in 27 of the 28 subjects. In one subject there was a 20 mmHg difference, and in this subject the intraarterial tracing (from the right arm) was compared to indirect measurements from the same arm just prior to the intraarterial measurement.

The auscultatory measurements were performed on the left arm simultaneously with intraarterial measurements in the right brachial artery. Among the obese subjects the indirect measurements were performed as double measurements in randomly selected order using both a standard cuff and a big cuff (dimension of bladder 12 × 23 cm and 14 × 45 cm, respectively). In the subjects with normal arm dimension only the standard cuff was used. The big cuff was made for this group of obese subjects and fulfilled the above mentioned criterion as regards length. In fact, however, even this cuff is too narrow to fulfil the width criterion (diameter +20%). But a broader cuff cannot be used in these obese subjects because of the conical shape of the arms. Therefore a 14 cm cuff was chosen as being the most suitable. The systolic pressure was

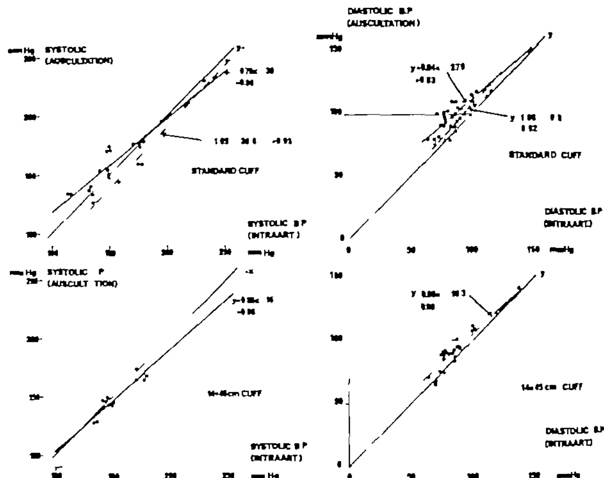


Fig. 1. Intraarterial systolic and diastolic BP compared to auscultatory BP in 28 very obese subjects with arm circumference 35–49 cm (● —) and in 21 subjects with arm circumference 22–34 cm (□ --).

d at the beginning of the Korotkoff sounds, and the diastolic at the cessation of the Korotkoff sounds (phase V). Using each cuff the mean of the double measurement was used in the calculations. The pressures were read to an accuracy of 2 mmHg.

We intended to use the "blind" manometer from the London School of Hygiene (13), but due to the slow deflation rate (2 mmHg/sec) during the whole measurement this manometer often resulted in severe discomfort for the patients and in unstable BP, especially when several measurements were to be performed. So in this study we used an ordinary mercury manometer. The measurements were carefully performed, so that the above mentioned deflation rate was used in the pressure range around the systolic and diastolic pressures, but between the systolic and diastolic pressures the deflation rate was about 5 mmHg/pulse beat.

Intraarterial measurements

These measurements were performed in the right brachial artery using a 19-gauge needle (o.d. 1.0 mm, i.d. 0.8 mm) in 13 of the patients with obese arms and 21-gauge needle (o.d. 0.8 mm, i.d. 0.5 mm) in the remaining 15 patients and in all subjects with normal arm dimension. A 15 cm nylon tube connected the needle to a capacitance transducer (Elema-Schoenander EMT 35). The tracings were recorded on a potentiometer writer (Mingograph 81). The linearity of the recording system was tested before and after the intraarterial measurement, using the following pressures applied with an ordinary mercury manometer connected to water-filled bottle: 0, 50, 100, 150, 200, 250, 200, 150, 100, 50 and 0 mmHg. The deflection of the potentiometer from these known pressures was for each patient read to an accuracy of 0.5 mm and plotted on mm-graphpaper. From this curve the intraarterial tracings could be read to an accuracy of 2 mmHg (1 mm on graphpaper = 4 mmHg).

The degree of damping was calculated using the square wave test (8). With the 19-gauge needle the degree of damping was 0.2–0.3 compared to 0.4 with the 21-gauge needle, i.e. both systems were slightly underdamped. No systematic difference was observed in the comparison between indirect and direct measurements with the two needles, and in the calculations the two groups of obese subjects were therefore combined.

During each indirect measurement of the BP on the left

Table I. *Auscultatory measurements of arm BP compared to intraarterial measurements in the br h al artery in the two groups of subjects*

	Arm circumference > 35 cm								Arm circumf rence [illegible] 34 cm			
	Cuff 12 23 cm				Cuff 14 45 cm				cuff 2[illegible] [illegible]3 cm			
BP (mmHg)	Auscul-tatory	Intra art.	Diff.	*t*-test[a]	Auscul-tatory	Intra art.	Diff.	[illegible]est[a]	Auscul tatory	Intra-art	Diff	[illegible]
Systolic												
Mean	16[illegible]3	155.5	+ 6.8	1.9	152.5	158.3	− 5.8	2.76	165 5	1 6 8	[illegible]	23
S.D	33 9	36.9	14 3		32.8	36 4	11.[illegible]		3 3	[illegible]0		
Range	{105	104	− 19[illegible]		{108	114	− 27	0 02	{110	1 6		[illegible] 0[illegible]
	{246	252	+ 34	$p > 0\ 05$	{240	258	− 19	$p > 0.01$	{ [illegible]40	[illegible]45		
Diastolic												
Mean	100 1	85 8	− 14.3	7.81	92.9	85 8	− 7 1	5.3	100 9	9 6		
S.D.	16.9	17.6	9 7		15 7	15.8	7 1		16 7	14 1		
Range	{62	64	− 8		65	64	− 10		3	64		[illegible]01
	{155	146	+ 35	$p < 0\ 001$	142	138	− 19	$p < 0\ 001$	1[illegible]	118		

[a] Method of paired comparison.

[illegible] markings were made on the intraarterial tracings, so that the intraarterial systolic and diastolic BP could be read at the exact moment of registration of the Korotkoff sounds.

Standard statistical tests were used in the calculations.

RESULTS

The individual BP values are plotted in Fig. 1 Among these obese subjects the auscultatory systolic BP was on an average +6.8 mmHg above the intraarterial BP (Table I) when using the standard cuff but 5.8 mmHg below the intraarterial value when using the broad cuff For the diastolic BPs the mean difference when using the standard cuff was +14.3 mmHg and +7 1 mmHg when using the broad cuff. Using the 12 23 cm cuff big interindividual differences existed between auscultatory and intra arterial BP This interindividual difference was reduced when using the 14 45 cm cuff as expressed in the reduction in the S.D (Table I) (14.3–11.2 mmHg for systolic and 9 7–7 1 mmHg for diastolic BP).

Among the patients with arm circumference 22–34 cm the difference between auscultatory and intraarterial systolic BP was on an average −11.2 mmHg (S.D 9 7), i.e. in this group the auscultatory measurement was significantly lower than the intraarterial measurements, while the auscultatory diastolic BP was +7.2 mmHg higher than the intraarterial (Table I).

It appears from Table I that the interindividual differences as expressed by the S.D were of the same magnitude in the non-obese as in th[illegible] [illegible] jects when the big cuff was applied [illegible] the [illegible] group.

In neither group was it possible to demo[illegible] a significant correlation between *arm* [illegible] and the difference between auscultatory and [illegible] arterial BPs (Fig. [illegible]), although the difference ter[illegible] to increase with increasing arm circumference [illegible] using the standard cuff in all subjects. Usin[illegible] the broad cuff on the obese subjects the regression lines (not shown in the Figure) were almost parallel to the abscissa (systolic BP $y = 0.09\,x - 9\ 7$ r 0.03 diastolic BP $y = 0.4[illegible]\,x - 9\ 4$ $r = 0.19$).

DISCUSSION

In several studies it has been shown that the accuracy of the auscultatory measurement of arm BP in *normal subject* is acceptable, especially when comparing the *mean* difference between auscultatory and intraarterial measurements. Table II presents the results from 6 studies of mainly normal subjects with normal BP and arm circumference. Apart from the study of Holland and Humerfelt (4) it will be seen that the indirectly measured systolic BP was on an average very close to the intraarterial pressure, while the diastolic BP (phase V) was 1 4–15 mmHg higher than the intraarterial values. The discrepancy between the results of Holland and Humerfelt (4) and the other authors is unexplained.

However when comparing the values from a s[illegible]

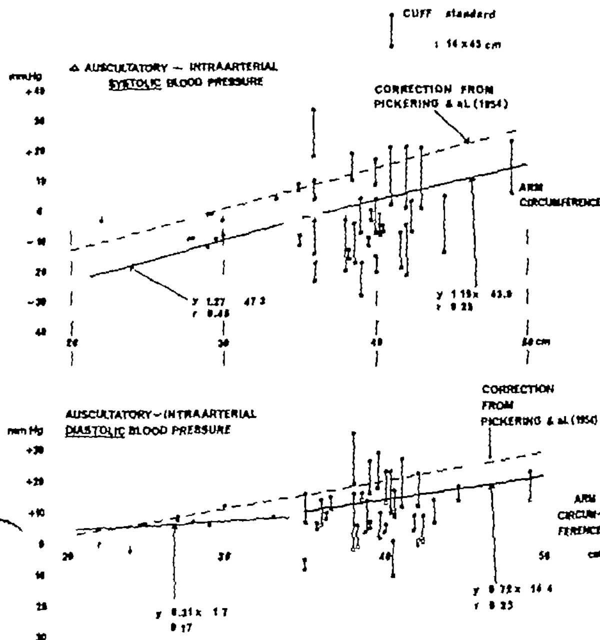


Fig. 2. Individual differences between auscultatory and intra-arterial BP in the two groups of subjects (arm circumference 22–34 cm and 35–49 cm. —— the regression lines for the two groups using the standard cuff ---- the correction from Pickering et al. (9) based on data from Ragan and Bordley (11) (systolic BP $y = 1.20x - 35.9$ $= 0.37$ diastolic BP $y = 0.87x - 15.5$, $= 0.32$).

patient the difference between indirect and direct measurements may be pronounced, as expressed by the S.D. of the difference. This S.D. lies between 5.7 and 14.0 mmHg for systolic and between 8.1 and 14.4 mmHg for diastolic BPs (phase V). These values are similar to those from our studies of normal subjects (Table I).

Earlier investigations of the validity of auscultatory measurement in obese subjects are shown in Table III. Most authors have performed the auscultatory measurement solely with standard cuffs, and in none of the studies has a comparison been made between the standard cuff and a broader cuff. Furthermore the number of patients was small.

Table II. *Auscultatory measurements of arm BP compared to intraarterial measurements in studies of normal subjects. Some of the materials include patients with hypertension and patients with obese arms*

Authors	No. of pats.	Age (y.)	Size of cuff (cm²)	Δ Indirect and direct BP (mmHg): Systolic Mean	Systolic S.D diff.	Diastolic (phase IV) Mean	Diastolic (phase IV) S.D diff.	Diastolic (phase V) Mean	Diastolic (phase V) S.D diff.
Ragan and Bordley (11)	40	19–55	13 23	− 0.4	12.2			+ 8.4	8.8
Karvonen et al. (5)	53	<20–>60	12 23 14 40	+ 0.5 − 3.0]	12.3 8.4	+17.7 +12.2	15.1 10.1	− 7.6 − 1.4	14.4 9.1
Holland and Humerfelt (4)	47	10–69	12 24	−24.6	14.0	− 5.3	13.9	−13.1	9.5
Simpson et al. (14)	24	23–66	12 23 12 35	+1.0 −3.7	10.4 5.7			+ 5.1 − 1.7	10.4 8.1
Raftery and Ward (10)	50	18–44	14 17.5	−5.4	11.6	+11.1	8.8	− 6.6	9.8
Forsberg et al. (2)	47	18–83	11.5 29	+4	12.1			+15	9.7

Apart from the results of Alexander et al. (1), which will be discussed, and of Simpson et al. (14) who presented only the mean differences using 8 different cuffs, the overall results were that, using the standard cuff, both the systolic and the diastolic BP will be considerably higher than the corresponding intraarterial BP. In the latter studies big interindividual differences were also observed. Trout et al. (15) and Forsberg et al. (2) pointed out that auscultatory measurements with the standard cuff placed on the upper part of the forearm will give values which are much closer to intraarterial measurements (Table II).

Alexander et al. (1) performed intraarterial measurements in 16 extremely obese subjects using a needle inserted in the brachial artery and compared these values to auscultatory measurements using a standard cuff just prior to the intraarterial measurements. These authors were, however, unable to find any great difference between these direct and indirect measurements (Table III). They therefore con-

Table III. *Auscultatory measurements of arm BP compared to intraarterial measurements in patients with very obese arms*

Authors	No. of pats.	Arm circumference	Size of cuff	Δ Indirect and direct BP (mmHg): Systolic Mean	Systolic Range	Diastolic Mean	Diastolic Range
Ragan and Bordley (11)	5	>35 cm	13 23 cm	+16	−2 +38	+22	+5 +32
Trout et al. (15)	6	>38 cm Cuff placed on the forearm	Width 13 cm	+55 + 3	+32 +102 −4 +30	+36 +10	+18 +66 +2 +20
Berliner et al. (1961)	10	Ponderal-index >4.25	13 20 cm	24	−5 +51	+19	−3 – +35
Alexander et al. (1)	16	Weight 243–487 p (mean 290 p)	Standard	+0.6	−23 +63 S.D. 20.9	−1.8	−24 +42 S.D. 16.3
Simpson et al. (14)	4	>35 cm	Mean using 8 different cuffs	+1.5	−4 7	0.5	
Forsberg et al. (2)	5	>40 cm Cuff placed on the forearm	11.5 61 cm	+34 +10	S.D. 14.6 S.D. 9.3	+30 +2	

Table IV. *Corrections for arm circumference to be applied to auscultatory measurements of arterial pressure based on data from Ragan and Bordley. From Pickering et al. (9)*

Arm circumference to nearest cm	Systolic BP (mmHg)	Arm circumference to nearest cm	Diastolic BP (mmHg)
15–18	Add 15	15–20	No correction
19–22	Add 10	21–26	Subtract 5
23–26	Add 5	27–21	Subtract 10
27–30	No correction	32 37	Subtract 15
31–34	Subtract 5	38–43	Subtract 20
35–38	Subtract 10	44–47	Subtract 25
39–41	Subtract 15		
42–45	Subtract 20		
46–49	Subtract 25		

cluded that a high reading by the cuff method in a very obese subject usually indicates a true systemic arterial hypertension. This conclusion is, as noted, in conflict both with previous measurements and our results. The explanation may be that the auscultatory measurements made by Alexander et al. were performed prior to the intraarterial measurement and not simultaneously.

In clinical work the corrections given by Pickering et al. (9) are well known (Table IV). These corrections are based on [illegible] ta from the study of Ragan and Bordley (11), who made the first simultaneous comparison betwe[illegible] auscultatory and intraarterial measurements us[illegible]g a standard cuff. Their material consisted of [illegible] subjects with arm circumference 23–[illegible]5 cm, but only 5 subjects had an arm circumfer[illegible] greater than 35 cm. Thus, from a statistical [illegible] of [illegible] these correction intervals for patients [illegible]th arm circumference greater than 35 cm cannot be accepted since only 5 patients were investigated. However, our regression line using the standard cuff is parallel with the regression line of Pickering et al. (9), although placed at a lower level (Fig. 2). Pickering et al. also emphasize that these corrections are useful when comparing groups of patients, but less significant when applied to a single patient.

In normal subjects and in subjects with moderately obese arms other authors have not been able to demonstrate a significant correlation between *arm circumference* and the difference between auscultatory and intraarterial BP (2, 4, 5, 10). These findings correspond to our results in these obese subjects, especially when using the big cuff.

In this study we could not demonstrate a correlation between the *level of BP* and the difference between auscultatory and intraarterial measurements, although the auscultatory systolic BP tended to lower than the intraarterial values in the hypertensive subjects. Corresponding analyses in hypertensive subjects with normal arm dimension have shown conflicting results (2, 3, 4).

The problems concerning the most suitable cuff for measuring auscultatory BP in these obese subjects are very similar to those relating to normal subjects. In spite of the fact that several authors and committees (5, 6, 7, 14, 16) have recommended that an ordinary arm cuff should fulfil the earlier mentioned dimensions (length equal to circumference of the extremity, width equal to diameter of the extremity +20%, i.e. in normal adults 12 35 cm), the standard cuffs from most manufacturers have not yet fulfilled these recommendations. An inquiry to five manufacturers concerning the size of "standard cuffs" gave the following results: arm cuff 12 23, 12.5 [illegible]4, 12.5 24, 12 32 and 12 22 cm, thigh cuff 11 60, 15 42, 18 40, 18 60 and 17 35 cm. If one performs arm measurement on very obese subjects using a "standard thigh cuff" it is clear that the values of the measurement will [illegible] most more on the manufacturer than on the cu[illegible]

The conclusion from our measurements [illegible] auscultatory measurement of arm BP in [illegible] subjects is inaccurate when using the [illegible] If a bigger cuff is used, the [illegible] be equally accurate as when measuri[illegible] normal subjects using the standard [illegible] the single patient the difference [illegible] and directly measured BP cannot [illegible] from arm circumference or [illegible] subjects in whom "cuff [illegible] intraarterial measurements are [illegible] prolonged antihypertensive [illegible]

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BUMETANIDE IN THE TREATMENT OF HEPATIC ASCITES

A Short and Long-term Study

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Abstract. A new diuretic agent, bumetanide, has been used in the treatment of ascites in 10 patients with cirrhosis of the liver. In a short-term study (7 days) the diuretic and natriuretic effect of the drug was demonstrated. Patients receiving 2 or 4 mg orally per day had highly significant negative sodium and water balance and an increased potassium excretion. In a long-term study (198–475 days) the effect on serum electrolytes and the toxicity of the drug were estimated in 6 patients receiving up to 6 mg daily. Except for a significant increase in standard bicarbonate, no change in serum electrolyte status occurred. Azotemia was only seen in two patients with high values prior to therapy. No changes in thrombocyte count, differential WBC, urine specimen or blood glucose levels were observed. One patient developed a Stevens-Johnson syndrome probably due to the drug. Bumetanide proved to be equal to other potent diuretics already in use in decompensated cirrhosis of the liver in respect to efficacy and side-effects.

Successful treatment of ascites in patients with hepatic cirrhosis depends on potent diuretics. However, these drugs carry hazards of electrolyte disturbance and coma, particularly hypokalemia is more frequent in patients with liver diseases than in other patients with fluid retention (4).

The present study was undertaken in order to evaluate the effect of a new diuretic, in patients with cirrhosis and ascites, in a short-term study in respect to water and electrolyte excretion, and in a more prolonged study (198–475 days) in respect to serum electrolytes, kidney and liver function, and possible toxicity.

PHARMACOLOGY

Bumetanide (Fig. 1) is derived from metanilamide, unlike furosemide and the thiazides, which are derivatives of sulphanilamide. A further difference is the presence of a phenoxy group (3). In man the metabolic fate of bumetanide has not yet been elucidated. In the dog it was not possible to detect any metabolites even after nephrectomy (3, 8). Diuresis starts within 30 min, reaches a peak at 1–2 hours and is virtually completed within 4–5 hours, as with furosemide, but the drug seems to be 40–100 times more active per weight unit (1, 8, 9). Serious toxic reactions to bumetanide have so far not been reported.

MATERIAL AND METHODS

The material consists of 10 patients with ascites due to cirrhosis of the liver of different etiology. In Table I clinical and laboratory data of the patients are presented. Impairment of tests of hepatic function was moderate or severe in all cases. None showed evidence of prior renal disease, but one (no. 10) had elevated serum creatinine (Fig. 3), possibly due to renal function impairment associated with hepatic insufficiency.

Short-term study

After a control period of one week, during which the patients were given a diet containing 50 mEq sodium and no diuretics, the patients were divided into two groups according to previous need for diuretic therapy. Group I included 5 patients (nos. 1–5) who had never required treatment with furosemide in doses greater than 80 mg daily. Group II consisted of 5 patients (nos. 6–10) requiring diuretic therapy with furosemide in doses exceeding 80 mg daily before admission. Group I patients then received bumetanide 1 mg b.i.d. for one week, while the patients in group II were treated with 2 mg b.i.d. All patients were given supplementation of potassium chloride in a slow releasing base 30–40 mEq daily from the beginning of the control week.

Long-term study

Six patients, three from each of the above groups (nos. 1, 2, 3, 7, 8, 10), were treated with bumetanide in doses 1–6 mg daily for 7–16 months, in total 65 months. Spironolactone, 25 mg q.i.d. was added in all cases. The patients were followed at monthly intervals in the Out-Patient Clinic.

Laboratory tests were carried out before, during and, when possible, after the treatment period. Initially wa

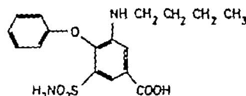


Fig. 1. Structural formula of bumetanide, 3-n-butylamino-4-phenoxy-5-sulphamyl benzoic acid.

balance, serum and urinary electrolytes and creatinine were recorded daily. Liver tests (alkaline phosphatase, alanine-amino-transferase, serum bilirubin, serum albumin, and prothrombin time) were followed at least twice weekly initially, later at monthly intervals. Besides routine determinations of Hb, ESR, leucocyte count, differential WBC, blood glucose, urine for sugar and protein were carried out.

RESULTS

Short term effect

The initial diuretic and natriuretic response to bumetanide treatment is seen in Fig. 2. During the control week all patients had a slightly positive sodium balance. A striking natriuretic response was observed during the first days of the treatment period, gradually decreasing. In group I the moderate sodium restriction and bed rest of the control period resulted in a negative water balance. A considerable diuretic response was observed in all cases within the first day of treatment, accompanied by a signif increase in potassium excretion. Except loss es, no changes of the clinical status took Development of hepatic coma or impairment renal function was not encountered.

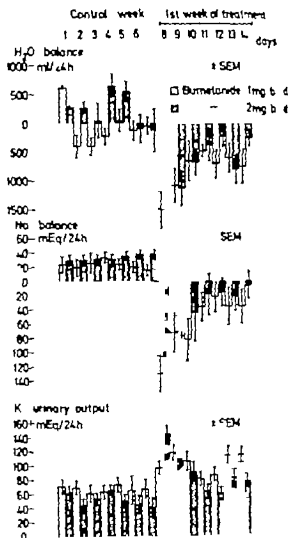


Fig. Immediate diuretic, natriuretic and kaliuretic response to diuretic treatment. Daily results indicated as mean values ± S.E.M. □ = group I, ⊡ = group II.

Table I. *Clinical and biochemical data of ten patients treated with bumetanide*

Normal values indicated within parentheses

Case no.	Sex	Age (y.)	Diagnosis	Ascites	Se-bilirubin (mg/100 ml) (< 1.0)	Prothrombin time (%) (85–115)	Se-albumin (g/l) (32.6–53.7)
Group I							
1	♀	32	Alcoholic cirrhosis	Moderate	3.9	61	26.5
2	♂	41	Alcoholic cirrhosis	Severe	5.3	45	18.7
3	♂	58	Alcoholic cirrhosis	Moderate	1.1	44	29.0
4	♂	60	Alcoholic cirrhosis	Severe	3.5	41	16.0
5	♂	63	Idiopathic cirrhosis	Moderate	0.4	85	36.4
Group II							
6	♂	47	Idiopathic cirrhosis	Moderate	4.1	37	22.4
7	♂	49	Idiopathic cirrhosis	Severe	0.8	45	30.6
8	♂	56	Idiopathic cirrhosis	Mild	1.8	76	27.0
9	♀	64	Idiopathic cirrhosis	Severe	1.6	—	23.9
10	♂	71	Alcoholic cirrhosis	Moderate	0.6	132	31.3

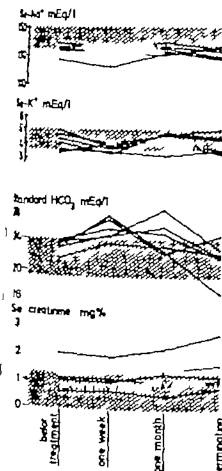


Fig. 3. Electrolytes and serum creatinine values observed in increasing intervals in 6 patients on long-term treatment.

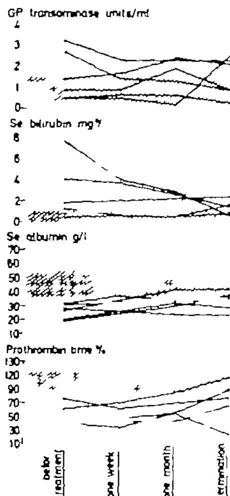


Fig. 4. Biochemical data reflecting the hepatic function observed in increasing intervals in 6 patients on long-term treatment.

Long-term effect

The effect on the serum electrolytes and serum creatinine is shown in Fig. 3. No significant changes in the serum sodium level were observed during treatment. All patients had low serum sodium either within or just below the normal range. Serum potassium was below normal values in one patient (no. 2) before treatment and remained below normal during the treatment. The serum potassium fell insignificantly during the first week of treatment ($p > 0.05$, paired t-test), but was otherwise stable. The standard bicarbonate level was within the normal range before diuretic therapy; when treatment was initiated a slight but significant rise was found ($p < 0.01$, paired t-test), reflecting compensated or slightly incompensated metabolic alkalosis. The serum creatinine was normal before treatment in all but one patient. In this patient a further rise was found, and in one other patient abnormal values were recorded during therapy.

Ascites was eliminated diuretically after 7–28 days of treatment and did not recur during the long-term treatment. In Fig. 4 some laboratory liver tests during long-term treatment are shown. In one patient (no. 7) deterioration of tests began after three weeks of therapy and did not improve during the treatment period (475 days) nor in connexion with discontinuation of the drug.

A Stevens–Johnson syndrome was observed in one patient (no. 8) after 21 days of treatment. It subsided after discontinuation of bumetanide. Muscle cramps in the lower extremities were a common complaint in three patients after 70–135 and ... days of treatment.

DISCUSSION

Bumetanide has proved effective in controlling edema in patients with heart disease of different etiology and in patients with nephrotic syndrome (1 9). In the present study the drug, given orally in doses 2–4 mg daily likewise resulted in natriuresis and diuresis equalling that obtained by using known potent diuretics in the treatment of hepatic ascites.

Hypokalemia and alkalosis, which may provoke coma in patients with cirrhosis, are occasionally aggravated by diuretic therapy (6, 12) Asbury et al. (1), in studying the effect of bumetanide, found an increased potassium excretion, but no hypokalemia or elevation of plasma bicarbonate. No information on potassium supplementation was given. In respect to potassium excretion and serum levels the results of Olesen et al. (9), their patients being on potassium supplementation, were in agreement with the present findings, while in respect to bicarbonate an elevation of plasma values, probably due to chloruresis exceeding the natriuresis, was observed. The present results are in accordance with those of Olesen et al., thus classifying bumetanide together with other potent diuretics in these respects.

The role of diuretics in precipitating or accelerating functional renal failure in advanced cirrhosis is still an unanswered question. The impression that this complication more frequently follows the vigorous use of more potent diuretics has not been established (11). The work of Lieberman and Reynolds (5) shows at, when this syndrome takes place during diuretic / it is most likely to occur in cases with ly high portal pressures The late stage of the w th increasingly resistant ascites, requiring higher doses of potent diuretics for its control, is likely to be the cause of the condition rather than the diuretic therapy per se. Asbury et al. (1) did not find any significant changes in serum creatinine after 8 days of continuous administration of 2 mg bumetanide daily Olesen et al (9) however treating 32 cardiac patients with bumetanide up to 4 mg daily found a slight but significant rise in serum creatinine after three months of therapy They ascribed this to a possible reduction in plasma volume, but pointed out that the evidence for decreased renal function might reflect the spontaneous course of the disease in the patients studied. In the present material the two patients who had aggravation of already high serum creatinine values received 4 and 6 mg bumetanide daily

Bumetanide was well tolerated by all except one patient who developed a dermatitis of the Stevens-Johnson type. It is well known that certain drugs may cause this type of multiform exudative erythema (2). In the present case a causal relationship is likely since the skin lesion disappeared after discontinuation of bumetanide. Muscle cramps in the calves, observed by Olesen et al. (9) in 2 of 32 patients, were found in the present material in 3 of 10 perhaps because the intracellular electrolyte and acid-base derangement is more serious in cirrhotic patients (7).

Bumetanide proved to be an agent of great diuretic efficacy The administration of the drug seemed to carry the same hazards as other potent diuretics in patients with poor hepatic function.

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PENICILLAMINE TREATMENT OF CYSTINURIA

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Abstract. Seven patients with cystinuria have been treated with D-penicillamine for 4–52 months. Excretion of free cystine in urine has been quantified regularly by ion-exchange chromatography. A specially short programme on an automatic amino acid analyser has been developed for this purpose. *The chelating properties of D-penicillamine are responsible* for loss of trace metals, and therefore zinc and copper in urine and zinc and ceruloplasmin in plasma were measured. Thus the dose of D-penicillamine can be reduced and trace metals can be substituted if there is any tendency to depletion. These corrections will presumably eliminate some of the drawbacks in *D-penicillamine treatment of cystinuria.*

Cystinuria is an inherited disorder affecting the transport of the amino acids cystine, arginine, lysine and ornithine in the epithelial cells of renal tubules and gastrointestinal tract. It is expressed clinically by formation of calculi in the urinary tract and constitutes 1 2% of all urinary stones in adults and 5% among children.

The poorly soluble cystine can be made more soluble by reaction between cysteine and D-penicillamine (8). The mixed disulphide is about 50 times as soluble as cystine. Since Crawhall et al. (7) introduced this treatment, several reports have confirmed the good results. However as many as 50% of the patients develop one or more complications like allergic reactions, fever arthralgia, proteinuria and the nephrotic syndrome (16), leucopenia, thrombocytopenia (17) and loss of taste (11). N-acetyl-D-penicillamine has also been used, which further increases the solubility of the disulphides ten times (21). But this drug has no other advantages. Because of the antipyridoxine effect of penicillamine the patients are supplemented with pyridoxine (13). The chelating properties of D-penicillamine are responsible for the increased excretion of copper and zinc and to a less extent of iron and calcium (3).

Treatment is directed at reducing the concentration of precipitable cystine in urine by increasing urine volume, alkalinizing with a large amount of sodium bicarbonate, or by the use of D-penicillamine. Usually this drug is given in large doses with*out quantitating cystine in urine. In some cases* cystine excretion has been determined by using an expensive and time-consuming full programme of an amino acid analyser

The purpose of this report is to show that the dose *of D-penicillamine can be reduced according to the* amount of free cystine in urine estimated through the use of a very short analysis programme (Fig. 1). The excretion of zinc and copper in urine and zinc concentration in plasma are followed together with *ceruloplasmin measurements, which give an idea of* the copper content of plasma. Trace metals are substituted if there is any tendency to depletion. These measures will eliminate some of the drawbacks in the D-penicillamine treatment of cystinuria.

MATERIAL AND METHODS

Of seven patients with cystinuria treated with D-penicillamine, four were female and three male, aged 23–53. The observation period at treatment was 4–52 months (Table I). Treatment with D-penicillamine (Cuprimine®) was started with a dose of 500 mg given at night. After a few days the dose was increased to 1 g. The fluid intake was about 3 l day. Previous sodium bicarbonate medication of 15 g was discontinued. Supplements of vitamin B 6 (Pyridoxine®), 80 mg day and vitamin B complex were given. Zinc was given as Solvezinc® 200 mg day. Stone formation did not occur in any of the patients and stones were completely resolved in two.

The therapy was usually started during a short stay at the hospital when hematological and electrolyte data, renal and liver function were studied. An i.v. pyelogram was also *performed at that time. Cystine and cysteine-penicillamine-*disulphide in urine were measured once a month, likewise the excretion of the trace metals. The hematological data, electrolytes liver function and presence of proteinuria

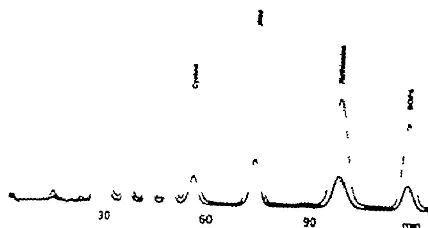


Fig 1 Elution profile of short programme for cystine and related compounds on an automatic amino acid analyser

assessed once week at the beginning of treatment and later once every third month Brand' nitroprusside reaction is used as screening test for cystinuria (4). All positi are run on high oltage electrophoresis at pH 1.9 and All final quantitations of cystine, arginine, lysine tine are done on Jeol 5-AH amino acid nalyser ll programme (14) During penicillamine treatment ntitativ determinations of cystine and penicillamine-cysteine-disulphide are routinely followed on an utomatic 70 min programme of ion-exchange chromatography developed for this purpose (14) Zinc nd copper in urine and zinc in plasma re analysed by atomic absorption (19). Ceruloplasmin is quantitated by electroimmunoassay (15). All other methods are standard methods at the Department of Clinical Chemistry

Table 1. *Clinical material at time of treatment with D-penicillamine*

Case no.	Sex	Age (y)	Months of treatment	Prophylactic clinical effect
1	♂	52	4	
2	♀	28	52	−
3	♀	53	15	−
4	♀	53	5	−
5	♀	52	6	+
6	♂	42	46	+
7	♂	47	40	

Stones resolved.

CASE REPORTS

Case 1

Male, 52 years. When 20 years of age multiple episodes of stone-passings occurred. From 1961 to 1963 seven spontaneous stone-passages took place. I 1963 left-sided pyelolithotomy was performed and this had to be repeated in 1964. In 1965 multiple concrements were removed gain from the left kidney In 1966 stone in the right ureter developed and serum creatinine level of 4.3 mg/100 ml was recorded. Renal function was restored after lithotomy When new staghorn stone in the left kidney was found in 1972, D-penicillamine treatment was started.

Case 2

Female, ged 28. I 1964 she was observed for suspected ppendicitis. During routine examination in 1968 proteinuria was noted, the I. pyelogram showing much staghorn stones bilaterally A left-sided pyelolithotomy was performed but concrement of 2 cm was left. During later right-sided pyelolithotomy multiple concrements were left, chemical analyses and mino acid analysis confirming the diagnosis of homozygous cystinuria. Therefore in Nov 1968 D-peni-

cillamine treatment was started and, since then, the course has been entirely uncomplicated. The residual stones in the kidneys have been completely dissolved and symptoms of stone formation have not occurred.

Case 3

Female, aged 53 (Fig. 2). During the 1940' she passed several stones every year. In 1961 a staghorn stone was removed from the pelvis of the left kidney and this was repeated in 1970. D-penicillamine treatment was started in Dec. 1971 but after three weeks taste was lost, so the drug was discontinued. During March–Dec. 1971 she had six spontaneous stone-passages. In Dec. of that year D-penicillamine was resumed. After two weeks of treatment the zinc level in serum became low and therefore zinc was given orally. Since D-penicillamine and zinc have been given, the course has been uncomplicated. No stone formation or hypogeusia have occurred.

Case 4

Female, aged 52 (Fig. 3). In the 1930' and 1940' she had multiple stone-passages but, since then, no stones have been formed. She was admitted to hospital because of hypertension. An i.v. pyelogram showed a small kidney on the right side with pyelonephritis. The pH of the urine was 7–8 without alkali treatment. Examination of the urine showed cystinuria of the homozygote type and she belonged to a large family in which several members had cystinuria. Treatment with D-penicillamine was started as prophylaxis. As shown in Fig. 3, the patient did not cooperate and, by quantitating the cysteine-penicillamine-disulphide regularly we could estimate exactly when she did not take the drug.

RESULTS

Case 2 has been treated with D-penicillamine for almost five years in combination with forced diuresis and alkalinization of the urine. During the last year sodium bicarbonate has been withdrawn. The stones have been completely dissolved and the drug has been effective in prophylaxis. The effect of the chelating properties of D-penicillamine is illustrated in case 3 (Fig. 2). There was massive excretion of copper and zinc in the urine leading to a low zinc level in serum. At the same time the patient complained of loss of taste. Zinc was given orally and taste was restored after some days. No low ceruloplasmin value was observed. Case 4 illustrates a patient who does not take drugs as prescribed. In our short analysis programme the cysteine-penicillamine-disulphide is followed regularly thus providing an easy method of monitoring the patient (Fig. 3).

DISCUSSION

The dosage of D-penicillamine is usually increased in order to reduce the urinary excretion of free cystine

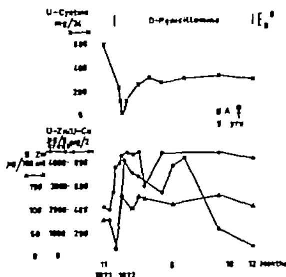


Fig. 2. Case 3. Effect of D-penicillamine on the excretion of cystine in urine. The chelating properties of the drug cause massive loss of copper and zinc in urine leading to zinc depletion, restored after giving zinc per os.

to about 200 mg cystine/g urinary creatinine (6). After five years experience the critical level for stone formation seems not to be as low as expected, because the urinary excretion in our patients remains at about 400 mg/g creatinine and has not given rise to stones. Many side-effects of the drug are likely to appear because of an unnecessarily high dose and too fast an increase in dosage.

Until 1963 the conservative treatment of cystin-

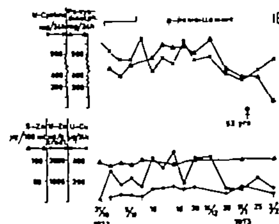


Fig. 3. Case 4. Advantage of regularly following the excretion of cystine and the cysteine-penicillamine-disulphide in order to check that the patient is taking

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uria consisted of forced intake of fluid and alkali in order to keep a high urine flow and urinary pH, which had to be maintained throughout the 24 hours of the day. In some patients this regime has been successful, but progress of the disease with repeated lithotomies, secondary renal damage and death in uremia in these patients is still very common (2).

D-penicillamine has also been used in the treatment of Wilson's disease (22), rheumatoid arthritis (1) and lead intoxication (9). But in most trials the incidence of side-effects of the drug is very high. The chelating properties of D-penicillamine are responsible for the increased excretion of zinc and copper in the urine. Hypogeusia has been reported but has been restored promptly after giving copper orally (11). There is, however, also a massive excretion of zinc, which in case 3 resulted in zinc depletion and hypogeusia. In severe burns a massive loss of zinc has been noted, leading to hypogeusia (5).

There is a complex autosomal recessive mode of inheritance. Harris et al. described in 1955 recessive and incompletely recessive forms with different urinary amino acid excretion patterns in heterozygotes (10). Rosenberg and coworkers have further classified the heterozygotes into types I, II and III on the basis of the intestinal and tubular transport of the four dibasic amino acids (20). Recent studies have shown greater genetic heterogeneity in renal and intestinal handling of amino acids in cystinuric patients (18).

The occurrence of a positive cyanide-nitroprusside ...tion in urine and a radiopaque concrement is ...quivalent to cystinuria. There is a need for ... investigation before treatment with D-peni...ine is started. The lower limit to the sensitivity of that r...ction is 75–125 mg/g creatinine. It is also positive in cases of heterozygote cystine-lysinuria, isolated cystinuria, homocystinuria, β-mercaptolactatecysteine-disulphiduria and general aminoaciduria. Thin-layer chromatography and high voltage electrophoresis are suitable media for the identification of cystine and the other three dibasic amino acids. If the patient is homozygous, all four amino acids are represented in high concentration. The magnitude of cystine excretion in 24-hour urine should, if possible, be quantified by ion-exchange chromatography before treatment with penicillamine. A special short 70-min programme on an amino acid analyser is of great advantage for following the cystine concentration. On our resin Jeol RC 2 the mixed penicillamine-cysteine-disulphide is eluted at the same position as valine. The amount of valine in urine is normally very low and the urine is in the case diluted ten times, so valine concentration is neglected. Therefore the mixed disulphide is also quantitated as a therapeutic control. Most amino acid analysers today have an automatic sample injector, so six to ten analyses can easily be done overnight. Treatment with D-penicillamine has replaced the traditional medical therapy in patients with cystinuria.

Which patients should then be treated with this drug? Large stones which could be obstructive must be removed surgically, but after operation D-penicillamine treatment should be started as prophylaxis. With patients who have been operated upon several times and so present difficulties when it comes to further operations, there is an absolute indication for treatment in order to dissolve the stones. In unilaterally nephrectomized patients, or where age or other complicating diseases prevent operation and where stone formation takes place, there is also an indication for D-penicillamine treatment. In cases with high stone-forming tendency after lithotomy or with spontaneous frequent stone-passings the drug should be given as prophylaxis.

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CORTISOL AND CORTISONE ACETATE IN PARENTERAL GLUCOCORTICOID THERAPY?

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Abstract Intramuscular cortisol and cortisone acetate have been administered to six patients with adrenocortical insufficiency Plasma cortisol determinations during the following four hours showed pronounced increase after injection of cortisol, but virtually no increase in five and only moderate increase in one patient after injection of cortisone acetate. It is therefore recommended not to use cortisone acetate in parenteral glucocorticoid therapy

An initial observation made in our department of the lack of biochemical and immediate clinical therapeutic success after i.m. injection of cortisone acetate to an Addisonian patient in a mild crisis focused our attention on parenteral glucocorticoid substitution therapy

It is usually recommended that cortisone acetate or cortisone acetate plus cortisol should be employed in parenteral glucocorticoid therapy for instance during preventive oral intake in Addisonian patients or as "steroid cover" during surgery in glucocorticoid-treated patients (1 5 6, 12). However the use of cortisone acetate may be questioned, as this steroid has been shown, although in a limited number of cases, to have variable and limited absorption after i.m. injection (14, 17 18)

In this study we show that m. injection of cortisone acetate results in minimal plasma cortisol levels, also compared to those obtained after m. cortisol.

MATERIAL AND METHODS

Six ambulatory patients were studied, four (nos 1–4) having documented Addison disease, one (no 5) Addison disease with partial adrenocortical insufficiency and one (no. 6) with apparently normal adrenocortical function, but previously with pronounced insufficiency following removal of an adrenocortical adenoma Age, sex and weight appear in Fig 1

Usual glucocorticoid substitution therapy was withheld 24 hours before the study At 8 a.m. the patients received an i.m. injection (gluteal region) of 90 mg cortisol (100 mg cortisol phosphate ester Actocortin Brd. Vernmehren & Ludvigsen, Copenhagen, Denmark) and on the following day contralateral i.m. injection of 90 mg cortisone (100 mg cortisone acetate, Merck, Sharp & Dohme, New York, USA). Blood was sampled from an i cannula placed in cubital vein before and 20, 40 60, 90, 120, 180 and 240 min after the hormone injection. Cortisol in plasma as measured by competitive protein-binding technique (10). Interference from cortisone is low by this method (3).

RESULTS

The time course of cortisol in plasma after cortisol and cortisone acetate is shown in Fig. 1 It is seen that within one hour after i.m. injection of cortisol, plasma cortisol increased to a maximum of about 30–45 μg/100 ml, which remained stable or slightly decreased during the following three hours. In contrast, i m. injection of cortisone acetate resulted in persistently low (<10 μg/100 ml) plasma cortisol values, *except in patient 4 in whom a moderate* increase of plasma cortisol was observed, compared to that obtained after i.m. cortisol.

DISCUSSION

The aim of exogenous glucocorticoid administration is to provide the patient with sufficient glucocorticoids. Of the natural glucocorticoids cortisol is the biological active hormone and cortisone must be converted to cortisol to mediate its glucocorticoid effect (8 16). The conversion of cortisone to cortisol after oral cortisone administration is deficient (9), oral cortisone being only two-thirds as effective as cortisol as a glucocorticoid agent (4, 20). This seems to be due to rapid metabolization of cortisone before conversion to cortisol (9). There is a paucity of clinical data on glucocorticoid activity after

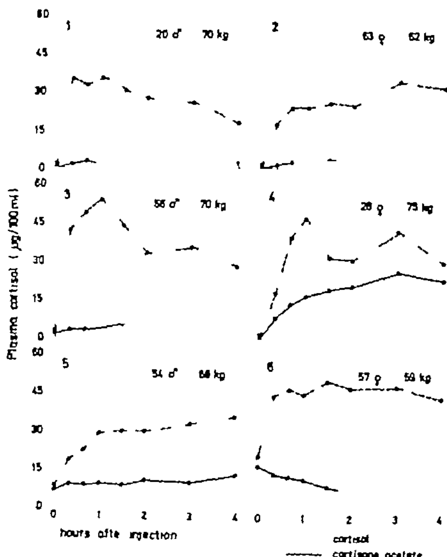


Fig 1 Time course of plasma cortisol after i.m. injection of 100 mg cortisol phosphate ester or 100 mg cortisone acetate.

jection of cortisone acetate Sporadic observations have been made which show unexpectedly low plasma cortisol levels (.., 7 14, 18). Likewise Plumpton et al. (17) found low plasma 11-hydroxy-corticosteroids in five glucocorticoid-treated patients receiving i.m cortisone acetate "cover" during surgery Newsome and Manalan (15) reported plasma cortisol levels of about 20 µg/100 ml in 4 patients studied after adrenalectomy but they used higher doses of cortisone acetate than Plumpton et al. and the present authors.

The relatively low cortisol values obtained after i.m. cortisone acetate are probably due partly to rapid metabolization of the absorbed cortisone acetate before conversion of cortisone to cortisol, and partly to inefficient and long-lasting absorption according to experimental studies (19). The result of the present study thus confirms that i m. cortisone acetate in usually recommended dosage is incapable of raising plasma cortisol levels sufficiently In contrast, i.m. cortisol results in a rapid increase of plasma cortisol. No major difference in efficiency exists between the various water-soluble cortisol esters, although the phosphate esters may give a slightly higher cortisol level (13).

It might be argued that long-time clinical experience tells that cortisone acetate constitutes satisfactory substitution therapy However this may be explained by the relatively high doses of cortisone acetate usually employed (6, 15). Furthermore recent studies have shown that stress-induced adrenocortical insufficiency is rare even when plasma cortisol is pathologically low (11).

In summary the secure and effective absorption makes it reasonable to use cortisol preparations for

i.v. or i.m. injection in parenteral glucocorticoid substitution therapy instead of i.m. cortisone acetate.

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SPLENOMEGALY ASSOCIATED WITH CHRONIC CONSUMPTION COAGULOPATHY

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Abstract. In a group of six patients suffering from various diseases accompanied by splenomegaly, low levels of factor V and fibrinogen have been observed apart from thrombocytopenia. No circulating fibrin degradation products (FDP) and fibrin monomers were present and anticoagulants did not correct the coagulation disorder. However, other findings, such as the presence of fibrin deposits in the enlarged spleen, and correction of the hemostatic defect after splenectomy strongly suggest local consumption of platelets and clotting factors in the enlarged spleen. The data obtained show that decreased concentration of clotting factors in patients with splenomegaly can be caused by local consumption alone. The absence of an elevated FDP level distinguishes our findings from those found in consumption coagulopathy accompanying diffuse intravascular clotting (DIC).

The hemorrhagic diathesis often observed in diseases of various origin complicated by splenomegaly can usually be attributed to thrombocytopenia. Pooling or destruction of platelets in the enlarged spleen is considered as the cause of the thrombocytopenia (13, 19). A more complex hemostatic defect resembling chronic intravascular coagulation has been described in patients with Gaucher's disease (29), fibrocongestive splenomegaly (12) and congestive splenomegaly of Mediterranean type (8).

In this paper we present six cases of splenomegaly in which some, but not all characteristics of consumption coagulopathy accompanying disseminated intravascular coagulation (DIC) were observed. As in two of these cases in which splenectomy was performed the coagulopathy disappeared, we suggest local consumption of platelets and clotting factors in the enlarged spleen as an explanation of this coagulation disorder. The disorder seems to be related to the enlargement of the spleen rather than to the cause of the splenomegaly. Next to the enlargement of the spleen there must be some other causal factor as we could not find this syndrome in all cases of splenomegaly. The selection for further investigation of these six cases was based on the observation of a relatively low ESR. Hence the incidence of this syndrome in association with splenomegaly is not known.

MATERIAL AND METHOD

Plasma was prepared by collecting nine volumes venous blood in one volume trisodium citrate 3.8% (w/v) and centrifuging for 10 min at 1000 *g*. Serum for the determination of fibrin degradation products (FDP) was obtained by collecting blood in a glass tube containing 50 U aprotinin/ml blood (Trasylol® Bayer). After two hours of incubation at 37°C and centrifugation for 10 min at 1000 *g* the supernatant serum was incubated for another 15 min at 37°C after addition of 0.02 ml thrombin (Topostasine Roche) 500 U/ml. The following determinations were done: blood platelets were counted (10), thrombin time was measured (0.1 ml thrombin 10 U/ml added to 0.2 ml plasma), levels of plasminogen (1), factor V (23), factor VII (18), factor X (2), prothrombin (30) and fibrinogen (25) were determined, FDP were measured in serum (7) and the ethanol gelation test was carried out (14).

Splenic tissue was obtained at splenectomy in two cases and immediately frozen in liquid nitrogen. Fibrinogen-related antigen in splenic tissue was demonstrated with the indirect immunofluorescence technique, using specific rabbit antihuman fibrinogen serum and fluorescein isothiocyanate conjugated horse antirabbit γ-globulin serum (11). The immunofluorescence determinations were performed by Mr A. van Rossum from the Department of Autoimmune Diseases, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service.

During treatment with acenocoumarin (Sintrom Geigy), the prothrombin time became 2–3 times as long as normal. Heparin treatment was controlled by means of the clotting time recorded with the thrombelastograph. *r*-value was between 30 and 75 min on 7 days and less than 30 min on 2½ days (case 1) and by the thrombin time, more than 180 sec for 2 days (case 2).

Table I. *Clotting factors and platelet counts*

Case no.	Diagnosis	Spleen weight (g)	Months[a]	Platelets[b] (10³/mm³)	Fibrinogen[b] (mg/100 ml)	Factor V[b] (%)	Factor VII[b] (%)	Plasminogen (%)	FDP (mg/100 ml)	Eth. gel. test[b]
1	Gaucher's disease	1 600	3	48–80 (4)	140–180 (8)	47–60 (8)	84–105 (8)	36–47 (5)	n.d.	d.
2	Polycythemia vera		1½	145–151 (3)	193–224 (7)	40–56 (6)	10[c] (1)	d.	<2 (2)	Neg (2)
3	Malignant histiocytic lymphoma	470	2	149–181 (3)	85–163 (9)	90–113 (3)	56–91 (3)	29–52 (3)	<2 (5)	Neg. (5)
4	Reticulum cell sarcoma	1100	½	37–102 (3)	60–67 (3)	72–80 (3)	70–108 (3)	32–68 (3)	<2 (2)	Neg (3)
5	Lymphoblastic sarcoma	3990	½	100–105 (2)	180–222 (2)	50–62 (3)	60 (1)	56–72 (3)	<2 (3)	Neg (2)
6	Malignant lymphoma		½	45–83 (3)	163–182 (3)	87–106 (2)	61 (2)	61–65 (2)	<2 (2)	Neg. (2)
Normal range		150–170		150–300	200–400	70–130	80–120	80–120	<2	Neg

[a] Interval between first and last determination.
[b] Maximal and maximal values of each determination. Number of determinations within parentheses.
[c] The patient was using acenocoumarin.
n.d. = not done.

CASE HISTORIES

Case 1

A 23-year-old man who has be[illegible] the subject of a previous publication (29). H[illegible] complained of troublesome nosebleedings during the last four [illegible] Local therapy gav[illegible] only temporary relief. On ad[illegible] p[illegible] cal examination revealed an enlarged spleen as [illegible] abnormality: the f[illegible] lower edge was palpable [illegible] the left costal margin.

Laboratory data [illegible] g/100 ml (normal range 13–17). [illegible] the usual liv[illegible] LDH, SGPT, SGOT, bili[illegible] alkaline ph[illegible] [illegible] including the disappearance rate [illegible] normal. The level of clotting [illegible] and pla[illegible] are given in Table I. Examination [illegible] sternal mar[illegible] the diagnosis of Gaucher's disease.

Treatment [illegible] coumarin (Sintrom, Geigy) for 12 days did not [illegible] *se in the number of platelets or the* levels of fibr[illegible] factor V. Neither did [illegible] continuous i.v. heparin in[illegible] ging from 40 000 to 160 000 U/day for 9½ days.

Because of th[illegible] tent bleeding tendency splenectomy was performed [illegible] ght of the excised spleen was 1 600 g. Typical Gaucher [illegible] ere observed in the splenic tissue and in that of [illegible] liver biopsy. The postoperative course of the levels of the clotting factors and platelet counts are shown in Table II. By means of the indirect immunofluorescence technique fibrinogen (or products having antigenic determinants in common with fibrinogen, i.e. fibrin or FDP) could be demonstrated in the spleen. These antigens were seen in [illegible] speckled distribution.

Case 2

A 71-year-old man, treated for polycythemia vera during the last 16 years, in the course of which ³²P had been administered four times. For some years he had been suffering from leg ulcers. The patient was admitted to the hospital because of thrombophlebitis. One year before admission three urate stones had been removed during cystoscopy and ever since that time he has been using allopurinol (Zyloric®, Wellcome) because of [illegible] high plasma urate level. On physical examination the liver was palpable 6 cm below the right costal margin. The splenic edge was palpable 6 cm below the left costal margin. No lymphadenopathy was observed. The right leg showed varicosis and superficial thrombophlebitis.

Laboratory data. ESR 1 mm/h, Hb 16.1 g/100 ml, WBC

Table II. *Effect of splenectomy on clotting factors and platelet counts of cases 1 and 5*

	Case 1		Case 5		
	Preoperative	Postoperative (1 mo.)	Preoperative	Postoperative (1 mo.)	Normal range
Fibrinogen (mg/100 ml)	150	40	180	390	200–400
Factor V (%)	50	80	62	120	70–130
Platelets (10³/mm³)	50	400	100	400	150–300
Plasminogen (%)	40	94	56	95	80–120

20 500/mm³ and plasma creatinine level 10.0 mg/l (normal range 6–12.5). All the usual liver function tests gave normal results. The levels of clotting factors and platelet counts are given in Table I.

Treatment with acenocoumarin (Sintrom® Geigy) during three weeks as well as with an injection of 2 500 U heparin followed by continuous i. infusion of 20 000 U heparin/day for two days did not produce rise in the number of platelets or levels of fibrinogen and factor V

Case 3

A 23-year-old woman, referred to the Department of Internal Medicine because of lymphadenopathy fever and extreme fatigue.

Physical examination revealed an emaciated ill-looking woman with temperature of 39.9°C. There was jugular, axillary and inguinal lymphadenopathy; the liver and the spleen were palpable just below the costal margins. On face, shoulders, arms and legs erythematous non-itching noduli, some brownish and of firm consistency were seen varying in size from some mm to 1 cm.

A biopsy was taken from one of these and the disease was diagnosed as malignant histiocytic lymphoma.

Laboratory data. ESR 12 mm/h, Hb 10.1 g/100 ml, WBC 2 800/mm³ and plasma creatinine level 6.0 mg/l. All the usual liver function tests were normal. Levels of clotting factors and platelet counts are summarized in Table I.

Therapy with 60 mg prednisone daily and 2 mg vincristine (Velbe® Lilly) once week was instituted. During three weeks the clinical condition improved, the temperature became normal and the size of the lymph nodes and spleen diminished. After some weeks, however there was an exacerbation and in spite of therapy with prednisone, cyclophosphamide (Endoxan® Asta) and nitrogen mustard the patient died 2½ months after admission. At autopsy innumerable enlarged lymph nodes and an enlarged spleen weighing 470 g were found. Extensive histiocytic infiltration was seen in the enlarged spleen, and generalized histiocytic infiltration in lymph nodes, skin, pancreas, kidneys and liver

Case 4

This patient, 40-year-old man, complained of fatigue and feeling unwell during the past year meanwhile he had noticed slow swelling of his abdomen. On admission we saw severely ill, emaciated man with remarkable pallor and temperature of 38.2°C. There was no lymphadenopathy the liver was palpable 10 cm below the right costal margin. The spleen was much enlarged, the lower edge reached the umbilicus. There was pleural exudate in the left hemithorax.

Laboratory data Hb 10.0 g/100 ml, WBC 1600/mm³ alkaline phosphatase level 25.5 U/l (normal up to 6.4), LDH 1000 U/l (normal up to 400), SGOT 162 U/l (normal up to 40), SGPT 165 U/l (normal up to 40), bromsulphthalein clearance: retention of 14.8% after 1 h (normal less than 5%), and plasma creatinine level 7.1 mg/l. The levels of clotting factors and platelet counts are shown in Table I.

Biopsies taken from the liver and the spleen yielded malignant lymphoma as most probable diagnosis.

Therapy with 60 mg prednisone daily gave an initial improvement, Hb content rose to 13.0 g/100 ml, the SGPT

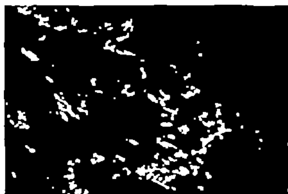


Fig. 1 Fibrin deposits in splenic tissue of case 5 demonstrated by means of the indirect immunofluorescence technique. (145).

dropped to normal level and the spleen seemed to diminish in size. However after some weeks relapse set in and despite therapy with vincristine the patient died 4½ months after admission.

Post-mortem examination revealed enlargement of liver (2 050 g), spleen (1100 g) and abdominal lymph nodes with extensive infiltration by polymorphic reticulum cell sarcoma.

Case 5

A 54-year-old man, referred to us by Dr T. D. Tan from the Department of Internal Medicine, Wesperplein Ziekenhuis, Amsterdam, complained of swelling of his abdomen, fatigue and perspiration during the night. The last week before admission he also suffered from pain in his left side and in the left shoulder

Physical examination revealed pallid-looking man with slightly enlarged liver (the blunt edge was palpable 3 cm below the costal margin). The spleen was very much enlarged and reached over the median line of the abdomen and into the pelvic cavity A splenic rub was heard at the left side.

Laboratory data: ESR 16 mm/h, Hb 9.7 g/100 ml, WBC 1 500–2 000/mm³ plasma creatinine level 8.8 mg/l.

The usual liver function tests gave normal results and the bone marrow showed no abnormality. The levels of clotting factors and platelet counts are shown in Table I

Splenectomy was performed and biopsy from the liver was taken. The weight of the spleen was 3 990 g. Histopathological examination revealed malignancy of lympho- or myelo-reticular type in spleen, liver and the lymph nodes of the spleen hilus. The postoperative course of the levels of the clotting factors and the platelet counts is shown in Table II. By means of the indirect immunofluorescence technique fibrinogen-related antigen scattered all over the spleen could be demonstrated (Fig. 1).

Eight months postoperatively the liver was found to be much more enlarged and cervical, axillary and inguinal lymphadenopathy had occurred. Examination of bone marrow and biopsy of an enlarged lymph node led diagnosis of lymphoblastic sarcoma. Therapy nation of cytostatic drugs gave occasional

Case 6

A 22-year-old Moroccan complained of perspiration and fever, diminished appetite and a swelling on his left lower leg during the last two weeks before admission to the hospital. At physical examination we found the temperature to be 39°C, a hematoma next to the left orbita and bloody crusts in nose and mouth. There were enlarged firm and tender lymph nodes in the neck and in the left submandibular region. The blunt non-tender lower edge of the liver was palpable 3 cm below the right costal margin, the spleen was palpable 5 cm below the left costal margin.

Laboratory data: ESR 50 mm/h, Hb 9.7-10.2 g/100 ml, WBC 2 000/mm³ and plasma creatinine level 4.9 g/100 ml. All usual liver function tests were normal. Levels of clotting factors and platelet counts are given in Table I. Bone marrow examination yielded the diagnosis of malignant lymphoma.

Therapy with 60 mg prednisone daily brought a dramatic improvement of the clinical condition. The lymph nodes as well as the liver and spleen diminished in size, the temperature became normal, Hb and WBC rose to normal values. The number of platelets, fibrinogen, plasminogen and factor VII levels returned to normal and a slight rise in the level of factor V was seen. After 2½ months the patient was discharged in good condition and returned to Morocco.

DISCUSSION

The degree and type of deficiency of the various coagulation factors in consumption coagulopathy are not uniform, but depend on the turnover of these factors in intravascular coagulation as well as on the ability of the individual to compensate for the increased utilization. In acute situations in which severe DIC is seen the laboratory confirmation of the process is rather simple. The presence of sub- or chronic intravascular coagulation is often difficult to detect and a number of laboratory tests, and perhaps an assessment of response to treatment may be necessary for the diagnosis.

To sustain the diagnosis of DIC the following criteria can be applied: 1) Low levels or increased turnover (24) of blood platelets, fibrinogen, prothrombin, factor V and factor VIII. 2) The detection of soluble fibrin monomers in plasma by means of the ethanol gelation test or the protamine sulphate test (17, 21), the latter being less specific (32). 3) Signs of secondary fibrinolysis: a low level of plasminogen and a large amount of circulating FDP. 4) A significant increase of the previously depressed coagulation factors and platelets during anticoagulant therapy. In some cases heparin stopped intravascular coagulation, whereas oral anticoagulants were ineffective (9, 20, 28). 5) The finding of fibrin and platelet aggregates somewhere in the organism after an episode of DIC.

Our cases fulfil the above criteria as follows.

In five patients there was a more or less marked thrombocytopenia. In four out of the six cases we found a definite fibrinogenopenia, whereas an elevated fibrinogen level was to be expected considering the state of disease of some of these patients; in three a reduction of factor V level was observed (Table I). The lowered factor VII level found in some of these patients can also be compatible with consumption coagulopathy (9).

The low levels of fibrinogen, factor V, plasminogen and factor VII cannot be ascribed to a deficient synthesis, since no sign of disturbed liver function was found in our patients, with the exception of case 4. Moreover, fibrinogenopenia caused by a defect in synthesis is only seen in cases of severe liver dysfunction. The return of the clotting factors to normal levels after splenectomy in cases 1 and 5 points to local consumption of these factors in the enlarged spleen and not to lowered production by the liver.

The ethanol gelation test was negative in all five cases in whom it was done, which means that the presence of circulating fibrin monomers could not be demonstrated.

The lowered plasminogen level present in all five cases in whom it was determined is a possible sign of activated local fibrinolysis; there was no elevation of the FDP levels in five cases and the normal thrombin time found in the sixth case also suggests a normal FDP level.

A possible explanation of the absence of FDP in the circulation might be local intravascular coagulation confined to the spleen without secondary fibrinolysis, due to the very low fibrinolytic potency of this organ. However, as in all cases the plasminogen level was lowered, a better explanation of this finding could be the immediate clearance of these products by the nearby reticuloendothelial cells in the spleen, which are known to have this function in DIC (5, 31). Whether treatment with an inhibitor of fibrinolysis might result in an increase of plasminogen level was not investigated.

In cases 1 and 2 treatment with acenocoumarin (Sintrom, Geigy) did not produce a rise in the number of platelets or the levels of fibrinogen and factor V. Moreover, after a continuous i.v. heparin infusion no change in these values was effected in either case. No explanation can be given for the fact that heparinization did not raise the deficient factors to normal values. It seems that heparin does not reach this local process. Other local processes,

such as giant hemangioma (Kasabach–Merritt syndrome), sometimes with splenic localization (6, 22) and arterial aneurysm (4), have been reported to cause chronic consumption coagulopathy.

In these processes, however, a raised level of FDP could be found (3, 4, 26), and therapy with coumarin and heparin or excision of the lesion corrected the coagulation abnormalities (4, 6, 15, 27).

With the indirect immunofluorescence technique we found fibrin (or products having antigenic determinants in common with fibrin, i.e. fibrinogen or FDP) in the excised spleens of patients 1 and 3. These antigens were seen in a speckled distribution (Fig. 1). Normal splenic tissue obtained at autopsy did not show these fibrin deposits.

Notwithstanding the negative ethanol gelation test, the absence of circulating FDP and the lack of reaction to heparinization, other findings, such as a low number of platelets and low levels of substrate clotting factors, the presence of fibrin deposits in the enlarged spleen and the return to normal values of the levels of the coagulation factors after splenectomy support the conception of local consumption of platelets and clotting factors in the enlarged spleen. The clinical implication of these data is that signs of DIC i patients with splenomegaly do not necessarily point to the presence of this syndrome, but that local consumption alone can be the cause of the disorder.

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PERICARDITIS FOLLOWING CARDIOVERSION

A Case Report

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Abstract. Pericarditis following DC countershock in a patient with atrial flutter is described and the literature on complications of cardioversion is discussed, with special emphasis on the possibilities of induced perimyocardial damage.

Reversion of supraventricular tachyarrhythmias to sinus rhythm by means of synchronized DC shock is nowadays a routine method in clinical practice. This method is considered more effective and—at the same time—less dangerous than traditional attempts at regularization by pharmacological methods (3 9 20, 21 23 27). On the basis of some ten years collective experience of cardioversion, the risk involved is, according to modern reviews, considered to be only slight (2, 12), and possible complications are thought to be well known.

Recently one of our patients presented transient symptoms of pericarditis in connection with cardioversion, which warrants a brief case report and a discussion of the pertinent literature.

CASE REPORT

Male, aged 71 treated for left pulmonary tuberculosis in the 1920's, otherwise previously healthy Since about 1960 he has had attacks of palpitation, usually lasting at most two or three hours and ceasing spontaneously Individual attacks in connection with infections were longer He was admitted to St. Erik's Hospital in 1963 and 1967 and diagnosed as having atrial flutter. Largely continuous digitalis treatment since about ten years; reacts with purpura to quinidine. ECG when the patient had sinus rhythm was quite normal (Fig. 1), heart size normal, never decompensated, normal BP Euthyroid. X-ray of the gall bladder in 1967 normal.

The patient was again admitted to St. Erik's Hospital in Oct. 1971, as he had suffered from palpitation and fatigue for over one month. The ECG showed atrial flutter with varying AV block, otherwise normal (Fig. 1b). Chest X-ray demonstrated only residual changes after the left-sided pulmonary tuberculosis, with parenchymal calcifications, pleural thickening and shift of the heart and mediastinum towards the left. Heart size and shape normal with relative volume 490 ml. General condition good, BP 145/90, irregular heart activity with rate of 100 min, but otherwise normal physical heart findings. N signs of recent infection or systemic disease. ESR 10 mm, WBC 9 500 with normal differential count. Routine laboratory examinations, including serum electrolytes and thyroid function tests, were normal.

Digoxin (Lanacrist® 0.375 mg 1) and verapamil (Isoptin 80 mg 3) brought subjective relief and normalization of the heart rate, but not reversion to sinus rhythm. After withholding digitalis for more than 24 hours, cardioversion was performed on Nov 16, applying normal routine procedure during short barbiturate anesthesia supplemented with succinylcholine. After two DC shocks of 100+200 Wsec the rhythm reverted to regular sinus, with normal ECG pattern. Awakening from the 5 min anesthesia uneventful.

On the same evening, bout 9 hours after cardioversion, the patient had chills, his temperature rose to 39.4°C, and he had deep chest pains on heavy breathing. No cough or dyspnea. Physical examination, by doctor on duty showed no abnormal findings. No change in therapy During the following day the fever gradually subsided and the chest pains disappeared. X-ray of the heart and lungs on Nov 17 did not demonstrate any additional change, but the ECG showed diffusely distributed current of injury as seen in perimyocardial irritation (Fig. 1c). On auscultation, however no pericardial friction rub was heard. During the next days the patient was again subjectively symptom-free, but the ECG showed progressive ST T changes indicative of current perimyocardial involvement (Figs. 1d and). Chest X-ray on Nov 19 unchanged with normal heart size and shape. The serum enzyme activities are shown in Table I. Cultures from the pharynx and expectorations were negative. Antistreptolysin titre 50, antistaphylolysin titre 1.0. C reactive protein + + + Immunological examinations, including antinuclear factor and antibodies against smooth and skeletal muscle tissue, on Nov 19 negative.

On Nov 21 atrial flutter recurred, causing cerebral embolism, with motor aphasia. In collaboration with neurological experts, anticoagulant treatment (coumarin) was introduced. During the following weeks there were repeated changes between sinus rhythm and atrial flutter and the latter became constant from the beginning of Dec. (Fig. 1f). The aphasia improved, and the patient was discharged in good condition after two months in hospital.

DISCUSSION

The potentially serious but rare complications of cardioversion, i.e. emboli in the lungs or in the

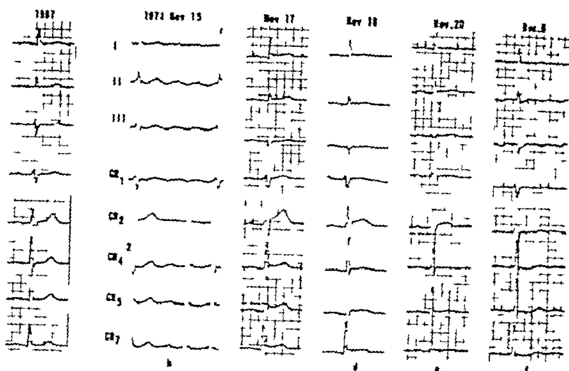


Fig. 1 ECG dev lopment

systemic irculat on and entricular arrhythmias (3 9 12, 1) are well known and will not be dealt with more closely

Pulmonary edema w thin 1 days after DC shock also been reported n numerous cases, first by ackos and M Donald n 1965 (19), and later by er authors (6 11 1.. 17 .5 27 28), who have so discussed the pathogenesis As a rule the pulmonary edema has been easy to treat by conventional methods and has only occurred in connection with cardioversions which ha e successfully resulted in sinus rhythm (or nodal rhythm in one case) Originally this edema was regarded as a sign of myocardial damage aused by the shocks, but—partly as a result of animal experiments—it is now attributed to a transient (days–weeks) defect in the contractile capacity of the left atrium, a delayed recovery as compared to the right side in spite of the return of a P wave in the ECG (5 20).

Table 1 *Serum e ms*

Enzyme	Date 15 11	17 11	18 11	19 11	11	11
GOT	18	46	[illegible]	34		[illegible]
GPT	22	46	[illegible]	34	[illegible]	[illegible]
LDH	155	12.	104			147
HBD	—	83			65	

Increase in one or more of the serum enzyme levels during the days following DC shock has been observed at an early stage as a common complication (18, 20 22, 27). The incidence varies in different studies from less than 10 (28) to more than 70 (1). Among the routine enzymes, especially creatine phosphokinase (CPK) and serum glutamate oxaloacetate transaminase (SGOT) activities are increased followed by lactate dehydrogenase (LDH). It has been shown that the released enzymes are derived from the thoracic skeletal muscles and are not indications of myocardial injury (7 10 13).

Thus neither the occurrence of pulmonary edema nor the much more common ncrease in the serum enzyme activities after cardioversion have been ascribed to myocardial damage nduced by electric shocks. However there seems to be greater uncertainty as regards the interpretation of the ECG changes observed in different investigations.

In 1963 Killip (8) reported transient (20 sec) elevation of the ST segment in a right precordial lead after two shocks of 200 + 400 Wsec in one patient of 62 examined. Oram and Davies (16) found in a study of 100 patients 12 cases of "injury current" which disappeared within a few seconds. In a case report by Sussman et al. (24) a marked ST elevation (current of injury) during 2 min after shocks of 100–200 Wsec is described. Turner and Towers (27) found among 50 patients 12 cases of "temporary electrical damage to the heart". Immediately after shock 3 patients showed ST elevation, and one patient an ST depression for less than 1 min, and in 8 cases there were significant changes in the T waves up to 4 days after cardioversion, most often in V_{1-4}, which was ascribed to the position of the shock electrodes. Mathivat et al. (14) found in 120 patients, immediately after shock, one case of ST elevation and 4 cases of ST depression. These changes were described as quite transient (from seconds up to a few minutes) and innocent. Reinikainen et al. (18) studied 63 patients, in 5 of whom immediate and rapidly passing ST depressions were observed, one patient presented a ST elevation in lead I for 5–6 hours. Of these 6 patients 4 also had temporarily raised serum enzyme levels. Faivre et al. (4) examined 80 patients and found that 11 had transient "courant de lésion" without sequelae. The changes were noted especially in V_{1-4} or V_{1-6}, where the anterior shock electrode had been placed, sometimes with an "image en miroir" in V. Morris et al. (15) found among 108 patients only one case of ST deviation lasting for 2 hours, whereas Szekely et al. (26) demonstrated 5 cases of transitory ST elevation in their 170 patients. Moreover in 2 other patients there was a T wave inversion for a few days, which was regarded as a sign of minor myocardial infarction. In 1967 Resnekov and McDonald (20) reviewed cardioversion complications in 220 consecutive patients. They reported 5 cases of T wave inversion lasting several days and one for at least half a year. These inversions were interpreted as probably indicating myocardial damage. Four of these 5 patients had been given shocks of more than 300 Wsec. In their study on 15 patients Åberg and Cullhed (28) observed transient ST changes in 3 but no T wave inversions were observed.

It seems that so far there has been no reports in the literature of observations either of ST deviations followed by T wave changes or of fever or deep chest pains related to breathing after cardioversion. Dreifus (3) states in his 1970 review that it now seems to be generally agreed that ST and T changes after DC shock are innocent. Q waves have never been noted.

In our patient a slight increase in the serum enzyme activities occurred on the day following cardioversion, but the same rise in serum glutamate pyruvate transaminase (SGPT) and SGOT values, combined with repeatedly normal LDH and α-hydroxybutyrate dehydrogenase (HBD) values, seems to distinctly contradict myocardial infarction. Pathological Q waves did not appear. There were neither clinical nor ECG signs of pulmonary embolism. The general distribution and the shape of the transient ST–T changes recorded on the days following the DC shocks indicate pericardial involvement. The clinical picture, together with the ECG development, appears to justify the diagnosis of acute pericarditis, possibly combined with myocardial involvement. A specific pathogenesis—infection, myocardial infarction, burns, immunological—seems unlikely, although coincidental subclinical viral infection cannot be definitely excluded. The subsequent clinical course, with relapse of the arrhythmia and probably a cerebral embolus, may have been influenced by the reported complication.

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INSULIN DEFICIENCY ASSOCIATED WITH HYPOGLYCEMIA AND GOOD GLUCOSE TOLERANCE IN HYPOPITUITARISM

Report of a Case with Acromegaly Developing Pituitary Apoplexy

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Abstract. A female patient with active acromegaly and latent insulin-deficient diabetes suffered pituitary apoplexy after which the markedly elevated plasma growth hormone decreased to non-detectable levels and did not respond to hypoglycemia. Simultaneously the clinical signs of acromegaly progressively subsided and the adrenocortical function became subnormal. However, in spite of an almost total absence of insulin secretion the fasting blood glucose decreased to slightly hypoglycemic range and the previously diabetic glucose tolerance was improved in spite of blunted insulin response. Adequate cortisone substitution did not influence either glucose tolerance or insulin secretion. These findings suggest that in the absence of growth hormone glucose assimilation may be rapid in spite of nearly complete lack of insulin.

Pituitary apoplexy is a well known complication of pituitary tumors including eosinophilic adenoma, in which it rapidly relieves the clinical and biochemical signs of acromegaly (3 4 17 18). However only few well documented case histories of acromegalic patients have been published in which the endocrine function has been studied in pre- and post-apoplexy states. We had recently an opportunity to carry out rather extensive endocrine studies in an acromegalic patient both before and after pituitary apoplexy The results are of interest mainly because the patient developed hypoglycemia and increased glucose tolerance in spite of an almost absolute lack of insulin. To our knowledge this peculiar combination has not been described previously

CASE HISTORY

The patient, 70-year-old woman, was examined at the Third Department of Medicine, University of Helsinki, on three occasions from Nov 1970 to Oct. 1971

First admission (Nov. 1970)

The patient was admitted to the hospital because of clinical signs and symptoms suggestive of acromegaly She complained of headache, excessive sweating, coarsening of voice and had noticed growth of hands and feet since several years. Clinical examination revealed characteristic acromegalic features with prominent nose and jaw macroglossia and thick and broad fingers. Her BP was 210/110 mmHg and the ECG showed atrial fibrillation with ventricular rate of 55/min. She was conscious and no abnormalities were found on neurological examination. The optic fundi and visual fields were normal. X-ray examinations of the skull revealed increased cortical thickness and enlarged frontal sinuses but a normal pituitary fossa. Typical acromegalic changes of the bones of hands and feet were found as well. The routine laboratory tests (Hb, WBC, ESR, serum creatinine) were normal.

Endocrine function. All laboratory data were consistent with a hypersecretion of growth hormone. Basal plasma immunoreactive growth hormone (IRGH) was markedly increased and showed an abnormal response to oral glucose load (Fig. 1). The glucose tolerance was clearly decreased and the plasma insulin response to oral glucose was within low normal range, indicating decreased β-cell function. Plasma inorganic phosphate and urinary hydroxyproline excretion were increased and the forearm skin was thickened (Fig. 2).

The patient was clinically euthyroid and, with the exception of slightly elevated protein-bound iodine, the indices of thyroid function were normal (Table I). Also the adrenal cortical function and the pituitary adrenal axis were normal. She was returned home to wait for pituitary cryosurgery

Second admission (April 1971)

Three months after discharge from the hospital the patient experienced an acute attack of severe headache and vomiting that lasted for four days. She was admitted to local hospital where the neurological examination and analysis of cerebrospinal fluid were negative. Three weeks after the acute event the patient was transferred to our unit again. She complained of increasing tiredness and was mentally depressed. The findings on physical examination were unchanged, BP being 170/90 mmHg. Routine laboratory tests (Hb, WBC, serum creatinine and electrolytes) were normal. The optic fundi and visual fields were normal. Pneumoencephalography disclosed a 5 mm displacement of the mid-line structures to the right. Cerebral scintigraphy was normal

literature, indicating that in acromegalic patients suffering pituitary apoplexy the plasma IRGH levels are either normal or decreased and often unresponsive to variations of blood glucose (4 15 16, 17 18)

A striking feature of the present patient was the alteration of glucose and insulin balance after the pituitary apoplexy Indeed, both can be accounted for by the lack of growth hormone. Evidently the patient had insulin deficiency of moderate degree already at the time when she was first seen with active acromegaly It is known from the studies by Luft and Cerasi (7) that acromegalic patients have either a normal glucose tolerance and hyperinsulinism or a decreased glucose tolerance (or diabetes) combined with a low and delayed insulin response. Growth hormone stimulates insulin secretion of prediabetic subjects much less than of normal persons (7). On this basis our patient can be classified as a prediabetic, in whom excessive endogenous growth hormone had caused an impaired glucose tolerance without being able to stimulate insulin release. On development of hypopituitarism and decline of growth hormone secretion to nearly zero the insulin secretion was further decreased and finally there was practically no detectable insulin response at all to i.v or oral glucose. In spite of this, blood glucose levels did not rise but, on the contrary a slight fasting hypoglycemia developed and the oral glucose tolerance improved. Thus the patient was severely insulin-deficient but still had a normal or even increased glucose disposal rate and no signs of ketosis.

The deterioration of insulin secretion may be due her to the natural course of the primary islet cell (prediabetes) or to absent or low plasma I It is known that after successful treatment of ly high insulin secretion is normalized while a low insulin response remains unchanged (7). Hypophysectomy in animals is followed by a decrease of insulin synthesis, amount and release in the islet tissue and this defect can be corrected by administration of growth hormone (1 8, 9). Low fasting plasma insulin levels are present also in human idiopathic hypopituitarism (5 14) and in patients with an isolated growth hormone deficiency (10 11). There are thus good reasons to believe that the exhaustion of insulin secretion was secondary to the IRGH deficiency Unfortunately this hypothesis could not be tested by administration of IRGH to the patient.

Also the decrease of fasting blood glucose and the improved glucose tolerance could be expected on the basis of pituitary hypofunction. Fasting hypoglycemia is known to occur frequently among patients with hypopituitarism (2) and it may form one of the presenting symptoms of pituitary apoplexy (16) as was actually the case in the present patient. However to our knowledge simultaneous assays of blood glucose and plasma insulin have not been carried out in these cases, except that Grunt et al. (5) reported low mean values for both glucose and insulin in five children with idiopathic hypopituitarism. On the other hand, sexual ateliotic dwarfs with an isolated growth hormone deficiency and poor insulin response regularly show a markedly decreased glucose tolerance (11). Taylor et al. (18) have reported a complete remission of diabetes without any change in insulin response in an acromegalic patient suffering pituitary apoplexy However this patient did not show a deficiency of insulin secretion, which was the characteristic and perplexing feature of our case.

These findings indicate that on absence of growth hormone the glucose utilization can occur at a very normal rate even in the presence of much reduced amounts of insulin. They also lend strong support to the view that growth hormone acts as a physiologically important regulator of glucose metabolism both directly and by influencing the secretion of insulin. However it remains unexplained why the patients with an isolated growth hormone deficiency exhibit a decreased glucose tolerance, while cases with a more general hypofunction of the pituitary often have hypoglycemia and increased glucose utilization. That this difference is not adequately explained by a deficient adrenocortical function in hypopituitarism is suggested by the failure of cortisol substitution to influence the glucose tolerance in the present patient.

Hypopituitarism is a usual consequence of pituitary apoplexy (18). In the present patient the low content of plasma cortisol and the weak response of urinary steroids to metyrapone suggested a deficient secretion of corticotrophin. After the apoplexy a normal level of circulating thyroxin was maintained and the patient remained clinically euthyroid. The content of plasma TSH was, nevertheless, low and unresponsive to stimulation with thyrotrophin releasing hormone (TRH). This situation is known to occur both in untreated acromegaly and after therapeutic intervention in the pituitary gland. The most likely explanation of the unresponsiveness to TRH stimulation seems to be a low TSH reserve in analogy with the situation observed after pituitary surgery

(6). Unfortunately urinary gonadotrophins were not determined in the pre-apoplexy state and therefore it is not known whether the hypogonadotrophism was a result of the pituitary apoplexy.

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Acta med. scand. Vol. 195 pp. 440, 1974

BOOK REVIEW

✓*Pharmacology of the endocrine system and related drugs: Progesterone progestational drugs and antifertility agents, volume II* Edited by M. Tausk. 538 pages. Sw cr 2.4 10 Pergamon Press, Oxford, England 1972.

This book is section 48 in the International Encyclopedia of Pharmacology and Therapeutics sponsored by the International Union of Pharmacology The volume gives an excellent review of the synthetic gestagens. Progesterone and its action on the female organism is dealt with in volume I of the same series. Chemistry pharmacology mode of action, metabolism and effects of the target organs are dealt with extensively for all synthetic gestagens used in clinical praxis. Indications for treatment and results are also given and discussed. In a special subsection the combination with oestrogens as used in the combined oral contraceptives is also covered. The reference list of the various chapters bears signs of having been closed at different periods. The reference list in some chapters ends in 1969 while other chapters are brought up to 1971 Another criticism is that the section on oral contraceptives is written by people with heavy personal commercial interests in the products.

The book appears excellent as a handbook for clinicians with an interest in the field, but also as an introduction for research workers.

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EDITORIAL

PREVENTIVE MEDICINE AND NATURAL DEATH"

The tasks of medicine may be defined as the relief of suffering, the restoration and maintenance of health and thereby the prolongation of life

Great emphasis has been placed in recent years on the aspects of health, sometimes in contrast to the relief of suffering. The more medical care has moved from an individualistic approach—the old patient-physician relationship and the fee-for service system—to centralized governmental systems, the stronger has been the influence of mass economics cost-benefit analyses rationalization, screening procedures and medical technology In this context—and in the face of vastly increased hospital costs due to expensive physical plant increased staff requirements because of specialization and reduced working hours (to be compensated for by hiring more staff) and last but not least, a substantial rise in salaries for the non-professional personnel—governments and hospital authorities are looking for less expensive forms for the delivery of medical care The substitution of the word health" for medical or medicine" is one expression of this policy health services health workers health centres etc.

It has been said that medical and med' cine are words which are less inviting than the positive word health" but this is certainly not true for most people The reason for this new speak to use an Orwellian term, is much simpler politicians and administrators are trying to convince themselves and the public that as good, or even better care can be bought at cheaper price w th less tax money through non-specialized preferably ambulant care and mass health screenings ma nly by means of laboratory robots. These items seem to be the main pillars upon which the case of preventive medicine" is built

There is of course nothing wrong with health" We are all for t the medical profession more than any other But the medical profession knows that health cannot be secured by any technical or social means In the main t is a gift bestowed upon a person at the moment of conception through the inheritance of good genes By the combined effects of healthy habits and good luck a person s health may be maintained for many years Good medical care can take the edge off unnecessary suffering or disability when diseases or accidents strike.

The more uccessful medicine—including preventive medicine—is the more people will survi e to old age Up to the present time old age in a great number of instances has carried with it an accumulation of disorders diseases and disabilities which—together with isolation reduced financial resources faulty memory loss of purpose and similar limitations—has led to an ever increasing demand for institutions with custodial care

Not only governments and medical administrators have expressed their concern about this development The no-longer-so-young generation has also observed the writings on the wall In an enqu ry to a fair number of Swedes over the last few years by one of our weekly magazines one of the questions was. How do you w nt to die A very big majority particularly among the males ndicated that they were hoping to pas aw y suddenly and w thout warning. What cardiologists all over the world rally to prevent appears to be precisely what many of those at risk seem to prefer

Viewed in a more general socio-economic perspective the situation for the elderly does not appear too bright either The gradual mo e from a three-generation society to four or even fi e generation society is pt to create increasing between the productive" (and repr

generation(s) and those who mainly will be at the receiving end Ironically it appears as if the industrial society—we have not seen any post industrial" society as yet and it is not so certain that it will ever materialize—is not capable of finding enough jobs for its citizens at least not jobs that are economically meaningful in terms of net profit or at such salary levels as are deemed acceptable by society and labour In such a situation the healthy pensioner may find himself with many years to life and probably a good deal of life to years" but nothing really to *do* until in due time general tiredness weakness and lassitude overtake him and his horizons shrink. Eventually he may then succumb to what a professor of geriatrics has labelled a natural death

There is nothing wrong with health Neither is anything wrong with "preventive medicine As applied to unborn newborn infants children adolescents young people mothers and bread winners it has always been undisputed straight forward and a blessing to individuals families and society From a cost-benefit point of view it has been a great success. But one ought perhaps to consider whether such a halo can be carried over without any reserve when the same concept is applied to the latter phases of life at least in countries where the life expectancy for those who have passed their mid-day extends to or beyond eighties as it already does If science is knowledge of consequences as René Dubos put it perhaps the scientists should examine possible consequences of preventive medicine on natural life as well as on natural death for the generation in question and for those who will follow after it

The point of view presented may appear unduly pessimistic However medicine has to be considered in a wide context and the more it becomes part of organized society the more it will be subject to the perpetual crises that seem to affect such societies In particular it will experience not only 'the slings and arrows of outrageous fortune" but even more the budgetary restraints put on activities that appear conventional" not very 'progressive and with unsatisfactory cost-benefit ratio Within these threatened areas are *both* the care of the suffering to the extent that they cannot easily be transferred by means of rehabilitation into the cadres of those 'gainfully employed" *and* the kind of intellectual endeavours that have been labelled study and research" inasmuch as their main aim is to satisfy curiosity rather than serve the purpose of production control"

Modern administrative technologies are creeping into the practice of hospital medicine They carry with them a risk of stereotyped behaviour and with it a temptation to superficiality in the midst of an abundance of means and measures theoretically available for diagnosis and treatment in the individual case

Preventive medicine may begin to claim recognition as a branch of science Clinical medicine in addition to being a science must maintain its quality of being an art.

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HYDRALAZINE INDUCED LUPOID SYNDROME

Biochemical and Immunological Studies

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Abstract A patient suffering from generalized scleroderma, who was on treatment with hydralazine (Hy) has been followed up biochemically and immunologically before during and after the onset of lupoid syndrome. A serum precipitation test, which was strongly positive in the acute phase of the condition is presented. No precipitation was obtained using sera of other patients with scleroderma or SLE or of controls. Low urinary values of sodium, potassium, calcium, phosphate and urea were noticed in relation to the crisis. A steep increase of the urinary output of acid mucopolysaccharides coincided while no significant changes in collagen metabolites were noticed. Urinary creatinine values were normal. Increased IgM and IgG were found in the solubilized precipitate of patient serum and the Hy-DNA comple after removal of DNA with protamine sulphate. In the kin biopsy obtained during the crisis, IgM and small quantities of complement were demonstrated by the direct immunofluorescence technique. By immunodiffusion the presence of precipitating antibodies against DNA was shown.

A lupus erythematosus-like syndrome (LS) can be induced by various drugs i.a. hydralazine (Hy) procainamide and chlorpromazine The syndrome occurs with highest frequency during long-term use of high doses of 1-hydrazinophthalazine (Hy Apresolin®) (1 9 11 19 20 21 22, 23 25 26) The condition occurs preferably in subjects who are slow acetylators of the drug (12 21). Although LS is considered a connective tissue disease clinically indistinguishable from systemic lupus erythematosus (SLE) the changes in the connective tissue components during the development of the condition have not been studied in detail Such information might help to understand the pathogenesis of the disease as well as its relationship with SLE It has been postulated that drugs inducing LS act by denaturing certain nucleoproteins and rendering them antigenic (22) Anti-desoxyribonucleic acid (DNA) and anti-Hy antibodies have previously been demonstrated in LS (13 14)

The question whether Hy may be linked to DNA and whether antibodies to the complex exist in the serum of LS patients has hitherto been unanswered. Serum precipitation, immunodiffusion and the direct immunofluorescent antibody technique applied to skin, together with biochemical studies practised before during and after treatment with Hy in a patient with LS are reported below

CASE REPORT

The patient was 30-year-old male with 10-year history of generalized scleroderma (acrosclerosis). With the rationale that Hy inhibits collagen biosynthesis (6), he was tentatively treated with Hy tablets (Apresolin®), 75 mg daily After about one year of treatment he started suffering from anorexia fatigue, joint pains, expectoration, diarrhoea and fever bout 39–40°C. H had skin petechiae, and despite withdrawal of Hy he developed an erythematous facial rash, anaemia, granulocytopenia, hypersedimentation, positive LE cell test, protein- and erythrocyturia, high-titred granulocyte-specific and low-titred tissue-unspecific antinuclear factor in serum. Blood cultures were negative

Upon the diagnosis of lupoid syndrome (LS) he was treated with betamethasone tablets (Celeston®) through about 3 months. The temperature normalized within few days, his habitual condition was regained, and, during the month following the laboratory tests normalized (Figs. 1–4).

LABORATORY STUDIES

Reagents

Desoxyribonucleic acid, highly polymerized DNA type I from calf thymus (Sigma Chemical Co. St Louis, Mo. USA)

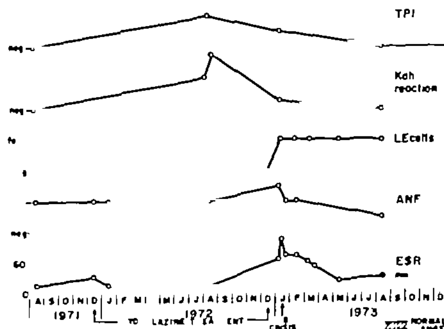


Fig. 1. Serum test before during and after the crisis.

Ribonucleic acid soluble RNA type III from yeast (Sigma Chemical Co. St. Louis, Mo. USA).

Heparin (Hep) and *hyaluronic acid* (HA) (gifts from Prof. K. Meyer, Yeshivah University, New York). Solutions containing 1 mg DNA, RNA, Hep and HA/ml water or 0.9% NaCl were prepared for immediate use.

Hydralazine HCl (E. Merck A.G. Darmstadt, Germany and Ciba-Geigy A.G. Basle, Switzerland) 12.5 mg were dissolved in 1 ml distilled water or 0.9% saline.

Procainamid (E. Merck A.G. Darmstadt) 22 mg dissolved in 1 ml distilled water.

Toluidine blue O solution (E. Merck A.G. Darmstadt) 1 mg/ml water.

Protamine-sulphat solution (Leo Pharmaceutical Products, Copenhagen, Denmark) 10 mg/ml water.

Rabbit anti-human IgG and IgM heavy chain specific (Dakopatts A.S. Copenhagen).

Rabbit anti-human IgG, IgM, IgA and complement C'3 conjugated with fluorescein isothiocyanate (FITC) (Dakopatts A.S. Copenhagen). The working dilutions were 1:20.

Coomassie Brilliant Blue (1137) (Edward Gurr Ltd. Michrome Labs. Cressex Industrial Estate, High Wycombe, Bucks, England).

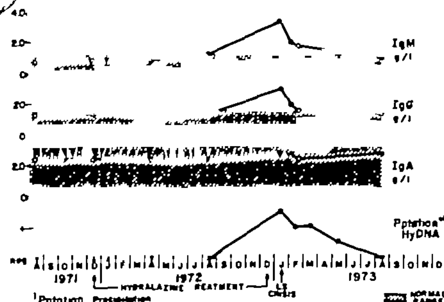


Fig. 2. Serum tests before during and after the crisis.

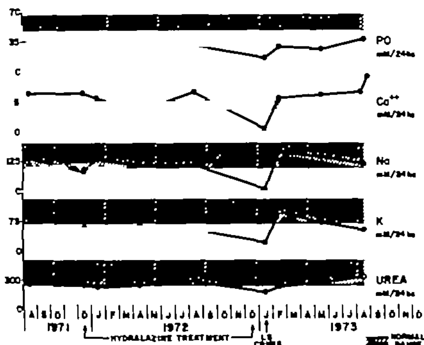


Fig. 3. Urinalysis before, during and after the crisis.

Denatured DNA: prepared by heating at 1 mg/ml aqueous solution of DNA at 100°C for 20 min and immediate chilling.

Hydralazine-native DNA complex (Hy-DNA): a mixture of equal volumes of Hy (12.5 mg/ml) and DNA (1 mg/ml).

Hydralazine-denatured DNA: mixture prepared as indicated for native DNA.

Hydralazine-RNA (Hy-RNA), *hydralazine-heparin* (Hy-Hep) and *hydralazine-hyaluronic acid* (Hy-HA): mixtures of equal volumes as indicated for Hy DNA. Similar mixtures of *procainamide* with the four anionic macromolecules DNA, RNA, Hep and HA were prepared.

METHODS

Urine collection. The patient received a collagen-free diet for 24 hours. Collection of 24-hour urine samples was performed during the day of the diet as well as the day before. Hydroxyproline (Hypro) in urine was assayed according to Kivirikko et al. (15) and Blumenkrantz and Asboe-Hansen (5). Acid mucopolysaccharides (Mcps) were assayed by the method of Blumenkrantz and Asboe-Hansen (4). Determination of Na, K, Ca^{++}, PO ––, urea and creatinine in urine were performed at the Department of Clinical Chemistry, Rigshospitalet.

Serum specimens. Venous blood was drawn during

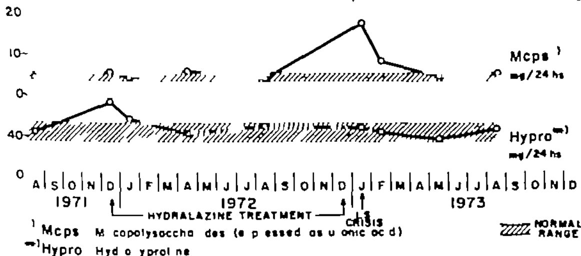


Fig. 4. Urinalysis before, during and after the crisis.

the course of treatment with Hy. Blood samples were also drawn from a control group consisting of 22 patients with generalized scleroderma, 1 with generalized scleroderma on Hy treatment, 15 with localized scleroderma, 20 with SLE, 1 patient with pseudoxanthoma elasticum, 6 on chlorpromazine treatment for mental disease and 1 patient under recovery from LS induced by treatment with procainamide for a heart disease. Twelve clinically normal subjects with negative ANF who were not taking any drugs were included in the control group. The blood samples were drawn on the same day as the urine was collected. The blood was allowed to clot and the serum separated by centrifugation at 000 rpm for 15 min. The serum samples were stored at −20°C.

Precipitation of sera with Hy DNA. One drop of serum was placed on a slide in the presence of one drop of Hy-DNA, and after 1 min the drops were mixed. Mixtures of the other anionic macromolecules and Hy were used in the same way. Aliquots of the sera of the LS patient as well as of 37 controls were inactivated by heating at 56°C for 30 min. Inactivated sera were mixed with Hy DNA and with mixtures of Hy and the other macromolecules as indicated above. Fresh sera of controls were added to inactivated LS serum plus Hy DNA.

Determination of IgG and IgM in precipitate of LS serum with Hy-DNA. Patient sera before and during the crisis of LS 150 µl, were mixed with an equal volume of Hy-DNA. The precipitate obtained was separated from the supernatant (S) by centrifugation at 3000 rpm for 15 min. The precipitate was washed 3 times with distilled water and recentrifuged. The washings were discarded and the precipitate dissolved in saline whereafter this solution was separated into two parts. 0.1 ml was analysed directly by immunodiffusion (saline solution of pellet, SSP). To the other 0.. ml ... 0.6 ml protamine sulphate solution was added. The precipitate formed was separated from the supernatant (S_1) by centrifugation at 3000 rpm for 15 min. The fraction S_1, SSP and S_2 were analysed by immunodiffusion.

Immunodiffusion assay. Immunodiffusion was performed according to Wadsworth (26). One per cent agarose in barbital buffer pH 8.6 was gellified under a matrix of plexiglass as described elsewhere (8). DNA, Hy and Hy DNA complex were dissolved in buffer and used as antigen. To determine the class of immunoglobulin anti-IgG and anti-IgM were used as antibodies in the immunodiffusion technique. The precipitates were stained with Coomassie Brilliant Blue.

Immunoelectrophoresis was performed with equipment from Dansk Laboratorieudstyr, Copenhagen. Precipitates of IgG and IgM developed by so-called rocket immunoelectrophoresis ( ).

Direct immunofluorescence studies. Two punch biopsies of skin (one during the other after the crisis) were obtained using ethyl chloride for local anesthesia. The biopsies were immediately frozen in liquid nitrogen and 4-8 µ thick sections were cut in a cryostat. The sections were air-dried for 15 min, washed in physiological saline for 30 min and incubated with a drop of conjugate in a moist chamber through 30 min. The samples were then washed 3 times in phosphate buffered saline (PBS) pH 7.. for 5 min and mounted in PBS containing 10% glycerol. The conjugates used were rabbit anti-human IgG, IgM, IgA and the complement C'3 conjugated with FITC. Control reactions were performed by blocking of the in vivo bound antibodies in the tissue sections by incubation with unlabelled antibody before the labelled antibody was added. Sections were examined in a Leitz Ortholux fluorescence microscope modified with a Tiyoda dark-field oil immersion condenser. A FITC interference filter was used as primary filter and mm glass filter (Schott and Gen, Germany) matched to fit the primary filter was used as secondary filter. An Osram HBO 200 lamp served as light source.

Serum complement C'3 of the samples was determined by Dr P. Halberg, Department of Medicine C, Copenhagen County Hospital, according to the method of Ogg (18).

RESULTS

When serum of the LS patient was mixed with Hy-DNA a filamentous and membranous precipitate appeared (Figs. 5 and 6). The serum precipitated only during the crisis and the following 4 months while no precipitate was formed with any of the control sera studied (Fig. 2). Increasing dilutions of the LS serum to 1:100 gave positive precipitation with Hy DNA while similar dilutions of control sera were negative. Neither the mixture of other anionic macromolecules and Hy nor Hy or DNA alone produced any precipitation.



Fig. 5. Precipitate obtained by mixing Hy DNA with serum of the LS patient during the crisis Jan. 1973 (8 and 9). Serum from Aug. 197? before the crisis, gave no precipitate (7).

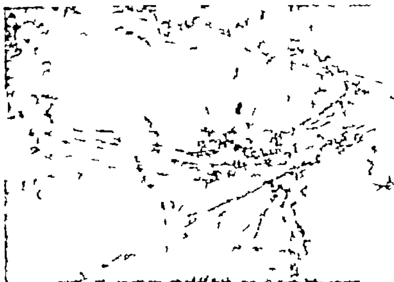


Fig 6 Filamento-membranous precipitate obtained by mixing serum of the patient during the LS crisis with Hy-DNA. Microscopic examination ×137

Sera of the LS patient during the crisis gave no precipitate when mixed with the Hy-denatured DNA complex. To eliminate the thermolabile fractions of complement the sera were heated to 56°C for 30 min. Inactivated serum plus Hy-DNA gave no precipitate. No precipitate was obtained when Hy and/or DNA were dissolved in saline. A precipitate appeared when the salt concentration was decreased by addition of distilled water. On the other hand the precipitate formed when serum of the LS patient was mixed with Hy-DNA complex in distilled water readily dissolved after addition of saline.

If the serum giving a positive Kahn reaction was precipitated with Hy DNA and the pellet separated from the supernatant, after being solubilized and made isotonic, both failed to give the Kahn reaction.

The anionic macromolecules Hep and HA prevented the precipitation reaction when added before the Hy-DNA complex. They also dissolved the already formed precipitate when added after Hy-DNA. Upon the addition of lower concentrations of Hep before Hy DNA partial precipitation was obtained. RNA did not influence the formation of the precipitate neither was a disappearance of the already formed precipitate observed.

No precipitate was obtained by mixing procainamide with DNA, RNA, Hep, HA, or with LS serum, neither was there any precipitation when the same mixtures were added to the serum of a patient with LS induced by procainamide.

The values of C'3 in serum of the LS patient before, during and after the crisis remained within the normal range of 140–180 mg% (18).

Acid Mcps give a metachromatic precipitate with toluidine blue (3). When a drop of Hy was mixed with a drop of an aqueous solution of Mcps, e.g. Hep or HA, no precipitate was formed, and the solution remained orthochromatic after addition of toluidine blue. If Hy was mixed with the metachromatic precipitate formed by Mcps and toluidine blue, the precipitate remained unaltered.

Immunodiffusion experiments showed the presence of DNA antibodies in the serum of the patient before, during and after the crisis, most distinct during the crisis. Hy gave precipitates not only with serum of the LS patient but also with serum of one normal person. Therefore the phenomenon was considered to be due to an artefact. The complex of Hy-DNA gave precipitation with serum samples of the LS patient before, during and after the crisis.

After separation of the precipitate of LS serum with Hy-DNA from supernatant S_1 the latter turned out to contain IgG and IgM (non-precipitable with Hy DNA). This was true both before and during the crisis. IgG was found in SSP and S_2. Traces of IgM were found in the latter fractions before and in increasing amounts during the crisis.

Immunoelectrophoresis of the fractions showed that the amount of IgG was unchanged, while the amount of IgM was increased in S_1 during the

Table I *Contents of IgG and IgM in different serum fractions during the crisis as separated by immunoelectrophoresis*

Fraction	IgG	IgM
S_1	Unchanged	Increased
SSP	Increased	Traces
S_2	Incre sed	Increased

crisis SSP and S_2 showed a slight increase of both IgG and IgM at the same moment. The content of immunoglobulins was considerably higher in the S_2 than in the SSP fraction (Table I)

In the skin sample removed during the crisis IgM and scarce amounts of complement could be demonstrated by the *direct immunofluorescent technique* The positive reactions were seen as a bright homogeneous band in the dermoepidermal junction. In the sample taken after the crisis immunoglobulins and complement could not be demonstrated The controls did not react

Upon determination of the *urinary excretion* of Na K Ca Po — urea creatinine Hypro and acid Mcps only the urinary values of acid Mcps (as expressed by uronic acids) were found to be increased even more than threefold during the crisis Hypro and creatinine showed no significant changes while all the other parameters studied showed a considerable decrease (Figs 3 and 4) The urine gave no precipitate with Hy DNA.

DISCUSSION

Our finding of a filamento-membranous precipitate formed by Hy DNA and serum of a sclerodermic patient suffering from LS and the lack of precipitation with sera of other patients with scleroderma and SLE, suggest a difference of specificity of the antinuclear antibodies formed in the three conditions The specificity of the precipitation was demonstrated by mixing other anionic macromolecules with Hy The fact that the reaction was still positive after the patient s recovery suggests that an immunological effect is in olved (13 14 15).

It is noteworthy that the precipitation reaction of the LS serum was positive only with the complex of native DNA and Hy while denatured DNA did not react.

The disappearance of the biological false positive seroreaction for syphilis (Kahn) of the serum after precipitation with Hy DNA deserves interest. A competition for a common site of attachment of the Hy DNA complex and the antigens used in the Kahn reaction may be responsible for the phenomenon

The lack of precipitation when inactivated serum of the LS patient was mixed with Hy DNA is in agreement with the complement-fixing activity of antinuclear antibodies induced by procainamide as reported by Kleiman et al (16)

Heparin seems to be able to react with the histone part of desoxyribonucleoprotein (DNP) removing DNA from the DNP complex (24) A relatively labile type of linkage as for instance in the Hy DNA complex could also be split by competitive displacement of DNA by Hep or HA Because an inhibition of their precipitation with toluidine blue takes place Hy seems to bind to acid Mcps.

As shown by direct immunofluorescence deposits of IgM and complement in the dermoepidermal junction during the LS crisis are similar to those observed in SLE Their absence during the remission of the condition after withdrawal of the drug and during the steroid treatment was coincident with the clinical improvement of the patient As scleroderma patients usually give no immunofluorescence the development of the positive reaction during the LS crisis in this patient can only be ascribed to LS induced by Hy (7).

The immunodiffusion studies showed the presence of precipitating antibodies against DNA in the serum of the LS patient in particular during the crisis At the same time an increase of IgM was recorded but this IgM was not precipitated after addition of DNA-Hy complex and might thus be directed to some other antigen(s). The question as to the presence of antibodies against Hy remains unsolved Originally we found a precipitation when in the diffusion experiments Hy was used as an antigen but the precipitation line was also given by a normal serum although it was weak and ill defined

The presence of IgG and IgM in the precipitate obtained after addition of Hy DNA to LS serum and the increase of these immunoglobulins during the crisis together with their release after remo al of DNA from the precipitate by addition of protamine sulphate clearly demonstrate the involvement of an immunological process during the

crisis It is noteworthy that the starting increase of IgM was coincident with the Treponema pallidum immobilization (TPI) and Kahn reactions

Our studies confirmed the assessment of Nickerson (17) of Na excretion in LS and demonstrated that other ions and catabolites were also reduced during the crisis. As they returned to normal values after the crisis we believe they can be used as parameters of the condition. Collagen metabolism seems unaffected while the excretion of acid Mcps is highly increased in the acute phase of the disease It is not clear whether the increased urinary output of these compounds represents an increased or an abnormal biosynthesis of the complex protein-Mcps, or is due to the release of proteases which can split the protein-polysaccharide complexes of the extracellular matrix. DiFerrante et al. (10) found the urinary excretion of acid Mcps increased in three of five patients with lupus erythematosus.

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CIRRHOSIS AND MALIGNANT HEPATOMA IN α ANTITRYPSIN DEFICIENCY

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Abstract Clinical and laboratory findings in 9 adults with homozygous (Pi^ZZ) α_1-antitrypsin (AAT) deficiency and cirrhosis of the liver are described. This clinical entity is characterized by rapidly progressive cirrhosis in non-alcoholics above 50 years of age resulting in severe portal hypertension and death in hepatic coma or bleeding. Concomitant emphysema, often subclinical, is usually present and malignant hepatoma is common. Histologically typical PAS-positive inclusion bodies are always demonstrable. Cholestatic features are not prominent. The entity is not associated with the HAA antigen, and serum "auto-antibodies" are lacking. In addition to AAT deficiency the serum protein analysis reveals the pattern seen in cirrhosis of other etiology but in most cases the IgM level was unusually high. The pathogenetic mechanism leading to cirrhosis in a minority at most 10% of adult patients with the Pi^ZZ phenotype remains obscure

The relation between genetically determined α antitrypsin (AAT) deficiency and panlobular emphysema is well established (7 27 29). Emphysema has been described also in children with this inborn error of metabolism (28). Since 1969 when Sharp et al (24) first reported cirrhosis in 10 children from 6 different kindreds with AAT deficiency this relatively common metabolic clinical entity in childhood (1 2, 22) has received wide attention. AAT deficiency in association with both cirrhosis and chronic obstructive lung disease in two sibs 11 and 1 years old, has recently been described (12). Single cases of cirrhosis in adults with Pi^ZZ phenotype have been published (5 11) as well as one case of cirrhosis with phenotype Pi^SZ (4). At examination of necropsy specimens of hepatic tissue from 13 subjects with AAT deficiency (Pi^ZZ) we found cirrhosis in 5 and fibrosis in 3. Most patients had emphysema and 3 also had hepatoma (3). The histology of the livers is characterized by an abundance of periodic-acid Schiff-positive globular material within the hepatocytes (3 23). Such material can also be demonstrated in carriers of the allele Pi^Z without affection of the liver

This paper summarizes the clinical and laboratory findings in 9 adults with severe AAT deficiency (Pi^ZZ) and cirrhosis

MATERIAL AND METHODS

The 9 patients were collected during 10-year period. All except 2 were personal cases. Only cases with an unequivocal cirrhosis (clinical and/or histological findings in necropsy or biopsy specimens (Menghini)) were included. The Pi^ZZ phenotype was confirmed in all cases by family studies or Pi typing, performed by Prof C.-B. Laurell by crossed antigen-antibody electrophoresis and starch-gel electrophoresis (8). Lung function tests were done in the way described previously (7). Dr N Egeflert Finspång, permitted us to study one of his patients. Dr B Lundh, Lund, placed case records at our disposal.

Agarose gel electrophoresis was performed according to Johansson (18) and electroimmunoassay of plasma proteins according to Laurell (20). The normal values used for various plasma proteins were those given by Hällén and Laurell (14). The serum was examined for α-fetoprotein by electroimmuno precipitation. The histologic procedures have been described previously (3). Liver function and serologic tests were performed according to standard procedures. Normal values are given in the Tables.

RESULTS

Table 1 summarizes the most important clinical findings. All patients were 50 years of age or more (mean 62) at the time of diagnosis of cirrhosis. In none of the cases could earlier episodes of icterus be traced. None had knowingly had neonatal hepatitis. None was a heavy drinker and two were even teetotallers. Liver cirrhosis had been diagnosed on clinical grounds in all the cases.

All the patients except one (no 4) had initially sought medical advice because al hyp

Table I. *Clinical findings in 9 patients with cirrhosi and AAT d ficiency*
+ = present − = absent

Case no	Age (y)[a]	Sex	Coexisting disease	Portal hyper tension	Cause of death	Malignant hepatoma	Emphy sema	Remark
1	83 (83)	♀	Rheum. arthritis	+	Kachexia	+	+	Non-smoker
	78 (78)	♀		+	Coma	+ (mult ple)	+	Non-smoker
3	69 (71)	♂	B defi ciency	+	Coma	+ (multiple)	+	Non-smoker
4	63	♂				−	−	Smoker
5	58 (58)	♂	Diabetes	+	Coma	+	+	Non-smoker
6	53 (53)	♂		+	Bleeding	−	+	Smoker Diabetes in elatives
7	53 (54)	♂	Hemoglo-binopathy	+	Bleeding	−	+	Non-smoker
8	5 (53)	♂		+	Bleeding	+	+	Smoker Diabetes in relatives
9	50 (50)	♀			Coma	+	?	Non-smoker Diabetes in relatives

[a] At diagnosis of cirrhosis age at death gi en within parentheses.

tension in which ascites was the dominant clinical symptom Spiders palmar erythema hepatic fe tor and/or flapping tremor had been observed on some occasion in all succumbed patients The dis ease was characterized by a rapidly progressive course with terminal complications in the form of coma or bleeding. It is seen from Table I that all the patients except one (no. 4) survived only years or less after the cirrhosis had been diag ..d Pruritus temporary was noted in only one ent (no 8)

Of the 8 patients who died 6 had primary liver carcinoma In 4 of them it was classified as hepatocellular (Fig 1 A) and in as cholangiocellular (Fig 1 B) Two patients (nos and 3) had multiple hepatoma. In only one of the patients (no) had hepatoma been suspected intra vitam.

One patient (no 5) had manifest diabetes mel litus Diabetes in first degree relatives was present in 3 other patients The fasting blood sugar and/or oral glucose tolerance tests were however normal in these 3 patients The glucose metabolism was also normal in a further 3 patients (not shown in Table I)

Macroscopically and microscopically all the livers showed cirrhosis with prominent nodular regeneration (Fig. ? A B) Cholestasis was seen in some cases but was rarely severe Steatosis was not prominent Siderosis was marked in one patient (no 7) who had an abnormal Hb (HbD

Table II. *Results of li er function t st*

Case no Normal range	Bilirubin total[a] (mg/100 ml) 0 ? 1	SGOT[a] (U) 10–40	Albumin[b] (g/100 ml) 4 5 ?	γ-globulin[a] (g/100 ml) 0 8–1 3	Alkaline phosphatase[a] (U) –8	Prothrombin acti ity[b] (%) 100	Cholesterol[b] (mg/100 ml) 150–250
1	0.3	148	9	1 7	1	60	180
	1	100	3 3	1 3	26	54	_35
3	4.3	97	4	3	9	47	190
4	0 4	5	4 3	3	4	100	340
5	4 6	_50	8	3 9	15	43	340
6	_3	10?	3	4 0	18	40	190
7	1 0	?90	3	4	17	30	
8	9.8	285	6	3 0	18	19	220
9	_1	45	8	1 8	13	45	150

[a] Highest. [b] Lowest value recorded.



Fig. 1 A. Case 8. Hepatocellular carcinoma with pleomorphic cells sometimes arranged in rings; htx-e ×178.

Punjab) in high concentration (50%) and signs of hemolytic anemia. All livers contained PAS-positive inclusion bodies of the type previously described (Fig. 3) (3, 13, 23). In no instance were such globules seen within hepatoma cells. Mallory bodies were never seen.

Most patients had emphysema. In several (nos 2, 3, 6, 7) it was clinically unrecognized. Severe anatomic emphysema with spirometric evidence of advanced obstructive ventilatory impairment was noted in cases 5 and 8. In case 4 there was no history of dyspnea and the results of lung function tests and X-ray examination were normal. Neither were there any signs of portal hypertension in this patient, the only one in the material who is still alive. In this material only 3 patients were smokers.

Table II summarizes the results of common liver function tests. Only the most abnormal values are given. Only one patient (no. 4) had a normal albumin value and most patients had polyclonal hyper-γ-globulinemia. Serum alkaline phosphatase activity was only moderately raised. The serum cholesterol was high in patients, one of whom

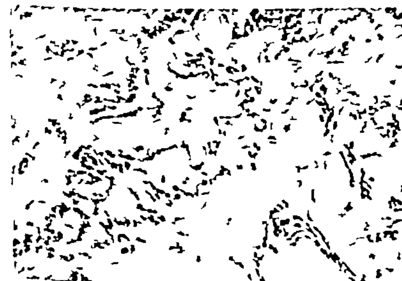


Fig. 1 B. Case 2. Cholangiocellular carcinoma. Acinocarcinomatous architecture; htx-e ×178.

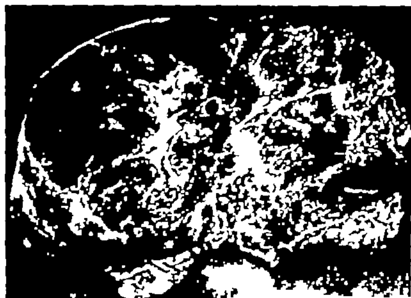


Fig 2A Case 8. Cirrhotic liver with diffuse nodularity (The car cinoma is not seen. It was found in the upper part of the right lobe after further cutting.)

(no 5) had poorly controlled diabetes The prothrombin activity was low in all patients with portal hypertension.

Table III gives the results of the serologic tests The antinuclear antibody test was sporadically positive in case 1 This patient had active chronic rheumatoid arthritis Smooth muscle and mitochondrial antibody tests were negative in 4 patients Hepatitis-associated antigen could not be detected in 7 patients. α-fetoprotein was detected in 1 patient. He had a malignant hepatoma.

In 7 cases examination of the plasma proteins was more detailed (Table IV). IgA and IgG values were increased in most patients but the relative IgM increase was larger than that for IgA and IgG Two patients (nos. 4 and 6) had very high IgM values The α_2-macroglobulin values were moderately raised. In one patient (no. 4) the value was very high The ceruloplasmin was slightly increased in most patients The orosomucoid showed no definite trend The haptoglobins tended to be low



Fig 2B Case 8 The histology of the liver with parenchymal nodules without normal lobular architecture separated by fibrous tissue bix-e 70

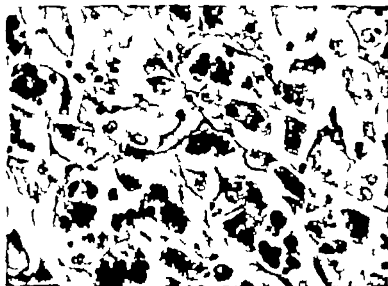


Fig 3 Case 8 Liver cells with PAS-positive diastase-resistant globules in their cytoplasm. PAS staining according to MacManus after diastase digestion of the section ×448.

DISCUSSION

In Pi^{ZZ} children an attack of jaundice in the first weeks of life is often followed by a free interval of varying length during which the definitive cirrhosis appears to become established. The early phase of jaundice which is generally referred to as "neonatal hepatitis" often has a prominent cholestatic feature and is histologically often associated with giant cell transformation (1 2, 22 24). It is not known what additional factors decide this course in at most 20–30% (1) of the children with ZZ homozygosity In contrast cholestasis seems to be uncommon in adults with AAT-associated cirrhosis As far as we know none of the patients in the present series had previously had any attack of jaundice and, judging from histologic and laboratory findings, cholestasis was not prominent. The alkaline phosphatases were only moderately elevated and hypercholesterolemia was uncommon (Table II) Of published cases of AAT-associated cirrhosis in adults prominent cholestasis was reported in only one This patient had advanced cholangiolar overgrowth within the portal triads and was regarded as a variant of biliary cirrhosis but the patient s history which contained notes about consumption of alcohol and chlorpromazine intake, had obscured these findings (11)

Detailed inquiry into the histories of the present patients excluded both alcohol and drugs as etiological factors of importance in the development of cirrhosis If the homozygosity were unknown the cirrhosis would surely have been classified as "cryptogenic" In representative liver cirrhosis series (15) the "cryptogenic group is as a rule dominated by women. In the present material the ratio between women and men was 1 2. But the size of the material does not warrant any definitive conclusions regarding the importance of sex in the development of cirrhosis in AAT deficiency The results of the serologic tests (Table III) underline another important difference between AAT associated and cryptogenic" cirrhosis In the cryptogenic group, as in chronic active hepatitis and primary biliary cirrhosis antinuclear (ANF)

Table III *Results of serologic t sts*

+=present or positive, −=absent or negative NP=not performed

ANF=antinuclear antibody SMA=smooth muscle antibody MA=mitochondrial antibody HAA=hepatitis-associated antigen, α-FP=α-fetoprotein

Case no	ANF	SMA	MA	HAA	α-FP
1	±	NP	NP	NP	−
2	−	−	−	−	−
3	NP	NP	NP	−	NP
4	−	−	−	−	−
5	−	−	−	−	+
6	−	NP	NP	−	−
7	−	NP	NP	−	NP
8	−	−	−	−	−
9	−	NP	NP	NP	NP

Table IV *Plasma protein pattern in 7 cases of Pi^ZZ-associated cirrhosis*

Case no	Orosomucoid (% of normal mean*)	Haptoglobins (mg/100 ml)	Ceruloplasmin (mg/100 ml)	α_2-macroglobulin (mg/100 ml)	IgA (g/100 ml)	IgM (g/100 ml)	IgG (g/100 ml)
1	140	125	55	315	0.37	0.30	1.3
[illegible]	150	100	45	310	0.16	0.19	0.9
4	100	78	45	730	0.20	0.7[illegible]	1.5
5	110	1	60	250	0.80	0.11	3.[illegible]
6	60	[illegible]0	35	[illegible]55	0.52	0.93	3.0
7	160	0	35	320	0.40	0.18	[illegible]0
8	60	20	40	[illegible]75	0.40	0.09	[illegible]0
Mean	111	48	43	351	0.35	0.35	[illegible]0
Normal mean ±S.D	100	87±37	37±7	120–310	0.19±0.07	0.08±0.05	1.11±0.[illegible]1

Refers to a pool of plasma from 1000 healthy donors used as standard.
Analyzed as Hb BC with a peroxidase technique

smooth muscle (SMA) and/or antimitochondrial (MA) antibodies (25) are common. Although liver cell specific antibodies have not been demonstrated and though these three clinical entities cannot be called autoimmune in the strict sense of the word the serological pattern suggests the occurrence of a pathogenetic mechanism common to all three entities. Like the 46-year-old patient with Pi^ZZ phenotype and cirrhosis reported by Cohen et al (5) our patients were seronegative, i.e. no such antibodies could be demonstrated. Although only 4 of our patients have been studied in this respect the results suggest [illegible] the cirrhosis in AAT deficiency is serologically unrelated to cryptogenic cirrhosis or chronic [illegible]tive hepatitis.

From a clinical point of view the picture in Pi^ZZ associated cirrhosis in adults is rather uniform (Table I): a fairly acute onset of portal hypertension in elderly persons (mean age 62 years) without heavy drinking or signs of liver disease in their history. Emphysema is common but often overlooked or misinterpreted. The prognosis is gloomy. Only one patient (no. 4) survived for more than [illegible] years after the diagnosis of cirrhosis. Coma and/or bleeding from varices is the most common cause of death but the high frequency of primary liver carcinoma contributes substantially to the poor prognosis (Table I). We have previously reported 3 cases of malignant hepatoma among autopsied patients with AAT deficiency (3). The present series of cirrhosis is larger and unbiased concerning malignant hepatoma, the only criterion for inclusion in the series being the presence of unequivocal cirrhosis in adult Pi^ZZ individuals. The demonstration of clinically unrecognized malignant hepatoma in 6 of 8 of the patients who died cannot be ascribed to chance but must be regarded as an expression of an unusually strong tendency to neoplastic transformation. The incidence is high even when compared with that found by Ohlsson and Nordén ([illegible]1) in a large autopsy material. Their figure (25%) for malignant hepatoma in cirrhosis is the highest so far reported (10). It is evident that cirrhosis in AAT deficiency should be considered a precancerous condition. With the possible exception of hemochromatosis (6) AAT deficiency in this respect seems unique among the inborn errors known to be associated with liver disease. Of interest in this connection is the report of 5 patients with cirrhosis and primary liver cell cancer with positive HAA tests ([illegible]6). The presence of Australia antigen has been discussed as one possible additional factor in the development of liver disease in Pi^ZZ homozygotes and has actually been demonstrated in a few cases ([illegible]) but not in most reports ([illegible]). It is seen from Table III that in our adult Pi^ZZ homozygotes with cirrhosis the HAA test was invariably negative. There is no reason to suspect that the hepatitis-associated antigen plays any significant pathogenetic role in this entity.

The results of standard liver function tests given in Table II fit in well with the pattern usually seen in patients with severe decompensated cirrhosis of any etiology. None of the values given are pathognomonic of cirrhosis in AAT deficiency.

The interindividual variation of the immuno-

globulin pattern in these patients (Table IV) is wide. Most patients had high levels of both IgA and IgG, the values being of the same order of magnitude as those reported in cirrhosis of other etiology. The IgM levels showed the largest relative increase. The mean value in 7 patients was 0.35 g/100 ml, which is twice as high as that reported in cryptogenic cirrhosis (14). The significance of this elevation is uncertain and it does not hold true for the individual case. The coexisting lung disease in these patients might also, in addition to the liver disease (19), influence the IgM level. A high concentration of this immunoglobulin has been reported in chronic obstructive lung disease in Pi^{ZZ} individuals (9).

Significant increases were found for both ceruloplasmin and α_2-macroglobulin (Table IV), but the levels were of the same order of magnitude as those reported in other types of liver cirrhosis (14). In no case was the ceruloplasmin level higher than 60 mg/100 ml. This finding is of interest in view of the very high level (141 mg/100 ml) reported by Ishak et al. (16) in one case of Pi^{ZZ} associated cirrhosis. Our data argue against an abnormal copper metabolism being involved in the pathogenesis of Pi^{ZZ}-associated cirrhosis.

The significance of high α_2-macroglobulin values in cirrhosis is unknown. This protein is synthesized in the liver, but high values are found also in patients with concomitant hypoalbuminemia. However, the highest value (730 mg/100 ml) was recorded in the only patient (no. 4) in this series with a normal albumin level and an apparently good prognosis. One might envisage a protective action of this protein in view of its capacity to bind and inactivate many proteolytic ferments such as leukoproteases with elastolytic or collagenolytic properties.

The occurrence of abundant cytoplasmic acidophilic bodies in the liver (Fig. 3), considered highly characteristic of AAT deficiency (13, 16, 23), has attracted much attention in the discussion of the pathogenesis of the liver disease in Pi^{ZZ} individuals. Such bodies occur in all Pi^{ZZ} individuals irrespective of age, but they tend to increase in number and size with age (3). They are also present, but less abundant, in Pi^{MZ} heterozygotes (1, 13). Their exact composition is not known, but they are surely antigenically related to α_1-antitrypsin (1, 13, 23). Whether liver damage is due to "toxic factors", such as excessive amounts of uninhibited proteolytic ferments, or whether it is a storage disease (1), additional factors must be sought to explain the occurrence of liver disease in only some Pi^{ZZ} individuals. The results of this series exclude alcohol, the HAA antigen, autoantibodies, abnormal copper or glucose metabolism as possible additional factors of importance.

Longstanding hypoxia must be considered one possible trigger mechanism in this connection. Most patients in this series had emphysema and consequently more or less pronounced hypoxia. On the other hand, the results of Gordon et al. (13) seem to exclude hypoxia as a factor of importance as all their 9 homozygotes had emphysema, but none had liver disease. However, their results were based on liver biopsy specimens from patients who were probably much younger than ours. Judging from our results, cirrhosis in adults with AAT deficiency is a complication of relatively high age and the risk of a patient developing this condition probably increases with age. Our working hypothesis to explain the liver disease in adults with AAT deficiency can thus be summarized: Owing to accumulation of inclusion bodies the hepatocytes are excessively susceptible to injury by hypoxia (and/or other unidentified factors), inducing a continuous though often subclinical damage to the hepatocytes. If the patient survives long enough, this damage combined with a hypothetical defect in the collagen metabolism due to abnormal handling of proteolytic ferments with collagenolytic properties results in fibrosis or cirrhosis. Exposure to excessive amounts of free protease may likewise be of importance in the development of malignant hepatomas in these patients. Cultured mouse fibroblasts have shown transiently altered growth behaviour on exposure to trypsin or plant proteases, reminiscent of the loss of contact inhibition observed in virally transformed fibroblasts (17).

It is impossible to give an exact figure for the frequency of cirrhosis in adult Pi^{ZZ} homozygotes. The 9 patients presented here constitute about 5% of all Pi^{ZZ} individuals registered at the Department of Clinical Chemistry, Malmö. It should, however, be observed that the majority of these individuals have not been studied with special reference to liver function and have not been subjected to biopsy. Many are dead and the autopsy records are not complete. Cirrhosis might

have been overlooked in several patients who had died from cor pulmonale and were not examined post mortem Our general impression is that the risk of cirrhosis is at most 10% a figure in good accord with estimations given by other authors (1).

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OCCURRENCE OF HEPATIC IMPAIRMENT IN WOMEN JAUNDICED BY ORAL CONTRACEPTIVES AND IN THEIR MOTHERS AND SISTERS

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Abstract. The occurrence of hepatic impairment has been investigated in women jaundiced by oral contraceptives and among their mothers and sisters. A comparison was made with matched control group, the investigated material thus comprising 129 matched pairs. It could be shown that the patients had experienced pruritus and jaundice during pregnancy more often than the controls. The patient group also showed higher incidence of hepatitis, gall stone symptoms and cholecystectomy. The patients' mothers had experienced pruritus during pregnancy to greater extent than their controls. The patients' sisters showed significantly higher incidence of pruritus during pregnancy and oral contraceptive treatment than their controls. Gall stone symptoms and cholecystectomy were also found more often in the group of the patients' sisters. The present study supports the hypothesis that genetic factors play part in the occurrence of jaundice during oral contraception and late pregnancy. The investigation also indicates that patients with jaundice due to oral contraceptive treatment must be considered as high risk patients with regard to their proneness to develop gall bladder disease.

The first suspicion that oral contraceptives might cause jaundice arose in 1962 when Perez-Mera and Schields (14) reported jaundice in a woman who had taken norethinodrel for about a month. In 1964 it was reported that serum transaminase activities and bromsulphthalein (BSP) retention increased in post menopausal women treated with oral contraceptives containing an oestrogen and a progestogen (4, 13). During the following years numerous reports on women jaundiced by oral contraceptives appeared in the literature, mainly from Chile and Sweden, and later but to a less extent also from other countries, e.g. Canada, Denmark, France, Germany and the USA (20). The patients had often previously had jaundice or pruritus of pregnancy.

Because the geographical distribution of the adverse reaction, and of reports on families with cholestatic jaundice during pregnancy or oral contraception in several members, it has been suggested that genetic factors play a part in the occurrence of the reaction (9, 10, 18). An earlier paper (20) contained data indicating that pruritus during pregnancy occurred to a greater extent among the mothers of patients than of controls, while there was no significant difference between the two groups of sisters.

The aim of the present study was to see whether women with jaundice from oral contraceptives, and their mothers and sisters, differed from control women who had used oral contraceptives for at least two years without adverse reactions and their mothers and sisters. The parameters looked into were the occurrence of jaundice and pruritus during pregnancy and oral contraception and the occurrence of gall bladder disease and other forms of jaundice. The main difference from the former study was that better matching between patients and controls was ensured and that the occurrence of gall bladder disease was studied also among mothers and sisters.

MATERIAL AND METHODS

A questionnaire was sent to 161 women with jaundice induced by oral contraceptives reported to the Swedish Adverse Drug Reaction Committee during the period Oct. 1965 June 1972. Only women with no other factors likely to cause jaundice, e.g. drug therapy injections, travel to foreign countries, in their recent medical history were included in the study. The questions asked were briefly whether and when they had been pregnant, whether jaundice or pruritus occurred during pregnancy and, if so, when and during which pregnancies. The women were also asked whether they had had gall bladder disease—if so when and whether cholecystectomy had been performed—and whether they had had other forms of liver diseases. The same questions were also asked about their mothers and sisters.

The same questionnaire was sent to 221 women who had received oral contraceptives from the National Association for Sex Education and the Department of Gynecology and Obstetrics, Danderyd Hospital, Stockholm, and who had answered an earlier questionnaire concerning oral contraceptives (3). According to their answers these women had

Table I. *Duration of oral contraceptive treatment in patients and controls (figures within parentheses denote %)*

Medication time (mo.)	No. of pats.	No. of controls
0–3	71 (55)	
3–6	19 (15)	
6–9	5 (4)	
9–12	15 (12)	
12–24	14 (11)	
24–48	5 (4)	68 (53)
49–72		36 (28)
>72		25 (19)
Total	129	129

used oral contraceptives without experiencing any adverse reactions for at least two years.

One hundred and forty-one patients and 210 controls answered the questionnaire. The remaining 31 women could not be traced. On receipt of the questionnaires the patients and controls were matched so that the parity was the same as as the year of birth ±2 years. If there was more than one control available the one was chosen whose mother's parity was closest to that of the patient mother. If there was still more than one control available, the one was chosen whose number of sisters was closest to the patient's. In this way we obtained 129 matched pairs. The matching was performed by an independent person who had no knowledge about the aim of the study.

Professor G. Eklund, University of Stockholm, Institute of Statistics, carried out the statistical analysis of the material.

RESULTS

The mean age of the patients was 31.2 years (range 9–53, median 30) and that of the controls 31.0 years (range 21–54, median 30). All had used oral contraceptives of the combined type. More than 50% of the patients developed jaundice within three months of the beginning of medication (Table I).

The patient group had experienced pruritus ($p < 0.001$) and jaundice ($p < 0.05$) during pregnancy more often than the controls (Table II). Also a past history of hepatitis, probably of the infectious type ($p < 0.05$), gallstone symptoms ($p < 0.01$) and cholecystectomy ($p < 0.001$) was found more often in the patient group than among the controls (Table II). The mean age of the patients when taken ill with gall bladder colic was 23.7 years (range 17–48) and of the controls 24.8 years (range 14–42). The mean age for cholecystectomy in the patient group was 25.5 years (range 17–48) and in the control group 27.2 years (range 21–43). The differences are not significant.

The patients mothers had experienced pruritus during pregnancy to a greater extent ($p < 0.01$) than the controls mothers. There was no significant difference between the two groups of mothers with regard to number of pregnancies, gall bladder colic or cholecystectomy (Table III).

The number of sisters in the patient group was not higher than in the control group nor was their number of pregnancies. The patients sisters showed a significantly higher incidence of pruritus during pregnancy and oral contraceptive treatment than their controls ($p < 0.001$). Gall stone symptoms were also seen more often ($p < 0.01$) and the incidence of cholecystectomy was significantly higher ($p < 0.001$) than among the controls sisters. There was no difference between the two groups with regard to the number of sisters using oral contraceptives (Table IV).

DISCUSSION

During the period Oct. 1965–June 1972 295 cases of jaundice suspected to be caused by oral contra-

Table II. *Result of questionnaire sent to patients and controls (figures within parentheses denote %)*

	Patients						Controls					
	Parity						Parity					
N	0	1	2	3	>3	Total	0	1	2	3	4	Total
Total	16	31	54	21	7	129	16	31	54	21	7	129
Gall stone symptoms	3	16	20	6	3	48 (37)	1	1	13	9	2	26 (20)
Cholecystectomy	0	11	15	5	3	34 (26)	0	0	5	4	2	11 (9)
Hepatitis in past history	0	2		1	2	7 (5)*	0	0	0	0	0	0
Pruritus during pregnancy	—	15	30	12	3	60 (47)	—	3	5	4	2	14 (11)
Jaundice during pregnancy	—	4	3	1	0	8 (6)	—	0	0	0	0	0

$p < 0.05$, $p < 0.01$, $p < 0.001$.

ceptives were reported to the Swedish Adverse Drug Reaction Committee. The 161 cases selected from this group for the present study were chosen because they showed the typical symptoms of severe pruritus, jaundice and nausea and had no known exposure to hepatotoxic drugs or hepatic infections. Some of the patients were taken ill after more than one year's use of the contraceptives, which is atypical for this reaction (16). They were, however, included in the study since the symptoms of the disease were typical and rapidly disappeared when medication was stopped. Although a great care was taken only to include patients with steroid jaundice, one cannot be absolutely sure that no jaundiced cases due to other causes are included in a material like this.

Retrospective studies can always be criticised because of the way in which the controls are chosen. The present investigation is no exception. It differs from the preliminary study (20) in that the controls were matched for parity and age. For practical reasons the matching was done after receipt of the questionnaires. This, however, could not have influenced the outcome of the investigation because of the strict matching rules decided on beforehand.

The higher incidence of jaundice and pruritus during pregnancy in the patients is consistent with earlier findings by Orellana–Alcalde and Dominguez (12) although they had no control series. It is clear though, that far from all patients with jaundice during oral contraceptive treatment react with jaundice during late pregnancy and vice versa. Rannevik et al. (15) investigated the hepatic effects of various sex steroids on 61 women who had had intrahepatic cholestasis of pregnancy. A transient increase of serum enzyme activity was found, but no increase in the concentration of serum bilirubin. There is no explanation why patients show this varying sensitivity to steroids. In their paper Rannevik et al. (15) suggest that, instead of simply contraindicating the pills for every patient with a history of intrahepatic cholestasis, one should closely follow their SGOT and serum bilirubin during the first months of treatment when they are most likely to develop jaundice. If elevated values are found and remain elevated, withdrawal of medication is indicated. To what extent this course of action could prevent jaundice during oral contraceptive treatment is not known. Some cases are reported to have occurred within one week after the commencement of medication (20), which indicates that the symptoms can devel-

Table III. *Result of questionnaire regarding the patients' and the controls' mothers (figures within parentheses denote %)*

N	Patients' mothers	Controls' mothers
Total	129	129
Gall stone symptoms	50 (39)	37 (29)
Cholecystectomy	31 (24)	29 (23)
Pregnancies	462	358
Pruritus during pregnancy	17 (13)	3 (2)
Jaundice during pregnancy	1 (1)	0

$p < 0.01$

very fast and these patients would therefore be difficult to catch before they develop their jaundice.

It is not known why women only sometimes respond with steroid jaundice. Maybe in certain women additional factors are needed. This is indicated in a paper by Ellström (5), who described two women who had taken oral contraceptives for one and two years, respectively, without any adverse effects and who developed cholestatic jaundice following cholecystectomy.

The mechanism behind the development of steroid jaundice is not known. Adlercreutz and Tenhunen (1) have suggested two possibilities: 1) an increased sensitivity of the bile-secreting function of the hepatocyte to steroids, or 2) a decreased capacity of the drug-metabolizing enzyme system in the liver cells, both of which could be genetic defects. It is debatable which of the two hormones in oral contraceptives, the oestrogen or the progestogen, is responsible

Table IV. *Results of questionnaire regarding the patients' and the controls' sisters (figures within parentheses denote %)*

OC = oral contraceptives

N	Patients' sisters	Controls' sisters
Total	130	112
Gall stone symptoms	34 (26)**	8 (7)
Cholecystectomy	26 (20)	5 (4)
Pregnancies	96 (74)	75 (67)
Pruritus during pregnancy	18 (14)	1 (1)
Jaundice during pregnancy	1 (1)	0
Users of OC	55 (42)	47 (42)
Pruritus when using OC	7 (12)	0
Jaundice when using OC	1 (2)	0

* $p < 0.01$ ** $p < 0.001$

for the reaction (1 7). Most authors, however are of the opinion that the oestrogens are mainly responsible for the reaction but that progestogens may play a part through metabolites with oestrogenic activity (7)

Steroid hormones can influence various factors involved in biliary excretion such as metabolic conjugation (8), hepatic uptake and secretion (11) and the permeability of the biliary tree (6). It has therefore been suggested that these substances might be capable of modifying bile composition and the biliary excretion of drugs and other chemicals (17). So far the problem seems not to have been investigated with the exception of studies on steroid induced changes in the hepatic clearance of BSP Both in animals and in humans it has been shown that the excretory capacity of the liver for BSP is reduced both during pregnancy and during oral contraceptive treatment (1). If there is severe impairment of the excretory capacity the patient develops jaundice, and intrahepatic cholestasis can be seen histologically In the present series liver biopsy was performed in 59 patients, all showing intrahepatic cholestasis and no inflammatory reaction.

The present study supports the hypothesis that genetic factors play a part in the occurrence of jaundice during oral contraception and late pregnancy Both the mothers and sisters of the patients developed pruritus, which is regarded as a milder form of the same disease (1), more often than the mothers and sisters of the controls.

It has been noted in a series of seven patients with jaundice from oral contraceptives that they had an unexpectedly high incidence of gall stones (19). This finding was confirmed by the present study which indicated that there is also a genetic predisposition. Both the mothers and sisters of our patients showed a higher incidence of gall stones and biliary attacks than did their controls. In this context it should be mentioned that Engström, Furhoff and Hellström (personal communication) have found a very high frequency of gall stones (51%) in a group of women who previously suffered from recurrent jaundice of pregnancy

Support for the idea that oral contraceptives can play a part in the development of gall bladder disease in susceptible patients is also found in an investigation from the Boston Collaboration Drug Surveillance Programme (2) Of 212 patients with gall bladder disease 65 (31%) were found to use oral contraceptives, while the frequency for 842 controls was only 20% The study furthermore showed that in women below the age of 35 years the frequency of gall bladder disease was significantly higher among those taking oral contraceptives for 6–12 months than among women who took them for longer periods. The authors suggest that in younger women those destined to develop gall bladder disease attributable to oral contraceptives tend to do so early in the course of therapy

Whether or not oral contraceptives should be prescribed for women with a history of other forms of hepatic disease or dysfunction is still not clear (7 15 16). In our series seven of the women had had infectious hepatitis before, but it is not possible to say whether this had any effect on the development of jaundice during oral contraceptive treatment since no details of the source of their disease are available. In general it is recommended that alternative methods of contraception should be used in patients with acute or chronic disturbance of liver function. If there is a history of acute liver disease in the past, then the oral contraceptive may be given if there is no evidence of disease at the time of treatment.

So far jaundice induced by oral contraceptives has not been reported to leave any sequelae. However the women experiencing this adverse reaction, as well as their sisters, must be considered as high risk patients with regard to their proneness to develop gall bladder disease

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MORPHOLOGY DIPEPTIDASES AND DISACCHARIDASES OF SMALL INTESTINAL MUCOSA IN CHRONIC RENAL FAILURE

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Abstract The function of the small intestine has been studied in 29 patients with chronic renal failure (CRF). Small intestinal biopsies were taken from 19 patient (11 haemodialysis (HD), 6 uraemic and nephrotic yndrome patients) and studied histologically and enzymatically (disaccharidases and dipeptidases). I half of these patients morphological changes i the form of longation of the crypts and invasion of plasma cells were found: 14 had decreased sucrase and maltase activities, 10 low glycyl-L-leucine and L-alanyl-L-proline dipeptidase activities only 2 had normal enzyme activities, 10 low glycyl-L-leucine and L-alanyl-L-proline the uraemic patients. Pancreatic function, tested in 5 patients, was normal. Increased faecal fat excretion was found in 4 of 14 low serum folate in 8 of 20 (before dialysis), and flat blood glucose curve was usually found in the peroral glucose tolerance test All these findings signify that disturbed function of the small intestine is not uncommon in patients with CRF This may be of importance when interpreting symptoms from the gut, especially when carbohydrate-rich diet is given.

Some disorders e.g., diabetes mellitus and chronic renal failure (CRF) may secondarily affect the gut. Little is known about the morphology and function of the small intestine in such diseases. Peroral biopsy technique now makes it possible to study this Recently Riecken (16) demonstrated morphological and histochemical changes in small intestinal mucosa from patients with CRF

An important part of the dietary treatment in CRF is peroral intake of essential amino acids (6 7). In recent years t has been shown that amino acids are better absorbed from the gut lumen if administered as peptides instead of free amino acids (14). It has also been suggested that the mechanism for transport of peptides across the mucosal cell is intimately connected with the peptide splitting enzymes (14)

In iew of these points we began an in estigation of the function of the small intestine including the morphology intestinal dipeptidases and disaccharidases in small intestinal biopsy specimens from patients with CRF

MATERIAL

The material consisted of 29 patients, 13 female and 16 male 19–69 years of age Eighteen had end-stage kidneys and were on egular haemodialysis (HD) treatment, 9 had uraemia, and nephrotic yndrome The clinical diagnosis of the different renal diseases was histologically verified at percutaneou renal biopsy and/or laparotomy (nephrectomy) or autopsy and was found to be: 6 patient with chronic glomerulonephritis (CGN) with nephrotic yndrome (NS) (PAD epimembranous and proliferative glomerulonephritis) 1 with lupus nephritis (SLE) 3 with chronic (non-obstructive) pyelonephritis (CPN) 5 with interstitial nephritis (IN), 3 with hereditary chronic nephritis (HCN) 2 with nephrophthisis (NT), with medullary cystic disease (MCD), with renal cancer (RC), postnephrectomy state 1 with retroperitoneal fibrosis and with nephrosclerosis (malignant hypertension)

On 21 occasion small intestinal biopsies er taken from 19 patients arranged in tw groups one of 11 HD patients (nos 1–11) (Table I) and one of 6 uraemic patients (nos. 1 17) and NS patient (nos 18 19) (Table II)

Haemodialysis group The patients undergoing HD treatment (twice weekly 8–10 hours with disposable parallel-flow artifical kidneys) received pproximately 0 9 g/kg b wt./day of high biological value protein. This maintained positive nitrogen balance and an acceptable serum albumin concentration in anephric patients. Each patient received 10 mg folic acid and vitamin B and C daily plus 1000 μg vitamin B_{12} monthly Bone marrow examination of the patients during HD show d no signs of megaloblastic erythropoesis.

The daily intake was rather constant for each patient and ut 55 cal./kg. None of

Table I *Clinical data on the HD group*

Pre=predialysis Post=postdialysis

Pat. no.	Sex	Age (y)	Diagnosi	Duration of dialysis (mo)	Serum urea (mg/100 ml) Pre	Serum urea (mg/100 ml) Post	Serum creat. (mg/100 ml) Pre	Serum creat. (mg/100 ml) Post	B wt. (kg) Pre	B wt. (kg) Post
1	♀	19	NT	20	120	20	11.5	3.3	46.1	45.8
2*	♂	51	MCD	16	82	31	13.9	4.7	58.4	57.7
3	♂	60	CPN	16	96	19	11.8	3.8	54.4	54.0
4*	♀	49	IN	14	124	1	11.1	3.0	67.9	66.6
5	♀	32	NT	11	107	16	14.3	3.5	48.9	48.[illegible]
6*	♂	57	RC	8	125	38	14.5	5.9	72.[illegible]	70.5
7	♀	53	IN	6	1[illegible]3	2[illegible]	10.0	[illegible].8	49.8	46.3
8	♂	29	MCD	5	86	[illegible]2	1[illegible]4	4.8	77.5	77.4
9*	♂	53	CPN	5	98	26	9.4	3.5	54.6	54.1
10	♂	33	HCN		83	31	13.8	5.9	68.8	68.3
11*	♂	42	CON	2	108	34	13.0	5.0	53.6	53.0

Nephrectomized.

the patients in this series had clinical signs of muscle wastage and did not develop peripheral neuropathy examined by EMG tests. Table I gives the pre and postdialysis values (average values for at least months of dialysis treatment) of serum urea and serum creatinine and body weight.

Uraemic and nephrotic syndrome group The patients in this group were not in an acute stage but were treated with a diet consisting of about 3 000 cal. including 35 g protein. They also received 500 mg methionine and tryptophan daily by capsule and vitamin B and C plus 5–10 mg folic acid daily if they had folate deficiency The NS patients received diet consisting of a high protein intake to compensate for loss of protein in urine These two patients (nos. 18 and 19) were the ly ones in the whole material who were treated with eroids (Prednison®) and immunosuppressive therapy (azathioprine) as well as diuretics (furosemide) combined with ldosterone inhibitors (spironolactone).

Reference group consisted of 32 adults and 12 children with histological normal mucosa and normal renal function (4)

METHODS

Laboratory tests Besides routine tests such as serum creatinine, serum urea and serum albumin the following laboratory tests were performed serum vitamin B_{12} (Euglena gracilis), serum folate (Lactobacillus casei) daily faecal fat excretion on 3-day specimens (9) and oral glucose tolerance test (30 g/m²) Pancreatic function was tested by determination of trypsin (5) and amylase (Phadebas Amylase test Pharmacia, Uppsala, Sweden) in duodenal aspirate before and after a test meal of water (2, 13).

Biopsy technique Biopsy of the small intestinal mucosa was taken at the duodenojejunal flexure with a hydraulic multiple capsule (15) under fluoroscopic control Three specimens were taken. one for morphological examination, one for assay of dipeptidase activity and one for disaccharidase activity The specimens used for enzyme assay were enveloped airtight in parafilm and stored at −20°C until analysis Stored thus the enzyme activities remain stable for months.

Morphological examination Each biopsy was oriented on millipore filter (11) and fixed in formaldehyde solution with short afterfixation in Bouin. After examination and photographing n a dissecting microscope the biopsy was serially cut into 5–6 μ sections. Alternative slides were stained with haematoxylin and erythrosin, with van Gieson stain and periodic acid Schiff according to McManus. The best oriented central cores of the specimens were used for assessment.

Table II *Clinical data on the uraemic and NS group*

Pat. no	Sex	Age (y)	Diagnosis	Serum urea (mg/100 ml)	Serum creat. (mg/100 ml)	4-h endogen creat. clearance (ml/min/1 73 cm²)
1	♀	55	IN	75	4.0	26
13	♀	49	SLE	160	7.0	7
14	♂	39	CON	88	9.0	9
15	♂	67	CON	220	12.5	7
16	♀	53	IN	154	13.7	7
17	♂	47	HCN	124	14.5	5
18	♀	69	NS	48	0.9	83
19	♀	52	NS	61	3.0	38

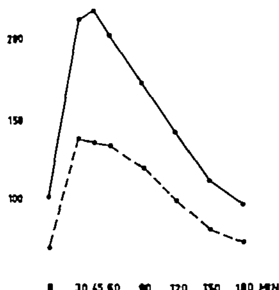


Fig. 1. Peroral glucose tolerance test in 12 HD patients aged 40–60 years (average curve ●- -●) and normal average curve in the same ages (●—●).

Special attention was paid to length of villi, elongation of crypts of Lieberkühn, changes of surface epithelium, and infiltration with plasma cells and neutrophil granulocytes.

Assays of enzyme activities. Dipeptidase activity was determined according to Josefsson and Lindberg (8) and Lindberg et al. (10) and disaccharidase activity according to Dahlqvist (3). Dipeptides used: L-alanyl-L-glutamic acid (ala-glu), L-alanyl-L-proline (ala-pro) glycyl-L-leucine (gly-leu), L-glutamyl-L-valine (glu-val), L-valyl-L-glutamic acid (val-glu). One unit of enzyme activity is defined as the activity hydrolysing 1 µmole substrate/min. Protein was determined according to Lowry et al. (12). Dipeptidase activity was expressed in U/mg nitrogen (17% of the protein) and disaccharidase activity in U/g protein.

RESULTS

Daily faecal fat excretion was investigated in 14 patients. All except 4 had normal values (≤5 g/24 h), i.e. one in the uraemic group had 6 g/24 h and three in the HD group 6, 6 and 13 g/24 h.

Serum vitamin B_{12} and folate were determined before the start of dialysis treatment, i.e. during the uraemic period. Serum vitamin B_{12} was normal in all 23 patients studied. Serum folate values were decreased in 8 of 20 studied, i.e. ≤3 ng/ml.

Fig. 1 shows the average blood glucose curve of 12 HD patients (aged 40–60 years) compared with the normal average curve in the same ages. The blood glucose rise is obviously lower in the HD patients. The same results were found in those aged below 40 and above 60 years, as well as in the uraemic patients of corresponding ages.

Table III summarizes the main morphological findings. Under the dissecting microscope most biopsies showed mainly leaf- or finger-like villi (Fig. 2*a*) but 3 biopsies showed ridging. In one of these pronounced ridging with convolutions was seen (Fig. 2*b*). Distinct elongation of the crypts usually combined with distinct Paneth cells in the

Table III. *Morphological findings in intestinal mucosa in patients with CRF*

	Total	Dialysis	Uraemia	Nephrosis	Remarks
Shape of villi in photograms					
Finger-like	2				See Fig.
Finger- and/or leaf-like	14	7	5		
Ridging	3	2	1		See Fig. *b*
Mucosal architecture in histological sections[a]					
Short crypts and regular fir-tree-like villi	6	3	2	1	
Crypts slightly elongated, villi less regular	6	3		1	See Fig. 3
Crypt length about 1/3 of villus height	5	4	1		
Crypt length 1/2 of villus height	2	1	1		See Fig. 3*b*
Plasma cells in mucosal stroma					
Scattered	7	4		1	
Small nests or slightly diffuse increase	7	5	1	1	
Distinct increase	5	2	3		
Total no. of biopsies	19	11	6		

[a] Villus height = distance between bottom of Lieberkühn crypt and tip of villus.



Fig 2 Small intestinal mucosa from two patients in the HD group. (*a*) Finger-like villi (pat. 2). (*b*) Pronounced ridging and convolutions (pat. 1) Magnification ×40

bottom of the crypts, was found in 7 biopsies (Fig. 3*a* and *b*) A few Paneth cells were also seen along the crypt wall Many mitoses in epithelial cells were found along the crypts and near the orifice. Heavy plasma cell infiltration was seen in five specimens Marked infiltration of lymphocytes or eosinophilic leucocytes was not seen in either the stroma or the surface epithelium.

The different abnormal findings were partly combined. four of the five specimens with many plasma cells showed elongation of the crypts but ridging was not seen in this group Two of these specimens, one in the HD group (pat. 10) and one in the uraemic group (pat 17) were from patients with HCN (Alport s syndrome) In one biopsy (pat 1) ridging and elongation of crypts were combined The abnormal findings were found in about the same frequency in both groups.

Duodenal juice was collected from five patients two in the HD group (nos. 3 and 10) and three in the uraemic group (nos. 14 15 and 16) In all the patients a normal activity of trypsin and amylase was found Fig. 4 shows the dipeptidase activities and Fig 5 the disaccharidase activities compared with the reference group About half of the biopsies had low activity on gly-leu and ala-pro (Fig. 4) whereas the other activities were less affected Only four patients had normal activities for all five dipeptides studied Among the disaccharidases sucrase and maltase were those most depressed 2/3 of the biopsies had decreased activities (Fig. 5) On the other hand only five biopsies had low





Fig 3 Histological appearance of two small intestinal biopsies with preserved villos structure but elongated crypt of Lieberkühn. (*a*) Biopsy from patient 7 in the HD group with slight elongation of the crypts. (*b*) Biopsy from patient 17 in the uraemic group with distinct elongation of the crypts and increased cellularity in the stroma.

lactase activity The f ve disaccharidase activities ere normal in four patients Only two patients (nos. and 19) had normal dipeptidase and disaccharidase activities. No difference in the enzyme activities was found between the HD and the uraemic groups

DISCUSSION

About half of the patients had morphological changes in the small intestinal biopsies In six patients these changes were rather pronounced (ridging, elongation of the crypts and heavy plasma cell infiltration) whereas in the others only slight changes were found These results are in close agreement with those of Riecken (16) He too found increased mitotic counts in villi and crypts which correspond with our findings.

Our earlier studies (1) show a close correlation between morphological and enzymatic changes in the small intestinal mucosa. Consistently the dipeptidase and disaccharidase activities were decreased in several patients However the enzyme activities were more affected than the morphology Thus, in only two patients were normal values of all activities found Of the various enzymes the changes were most pronounced for sucrase and maltase and not, as might be expected for lactase (1). This finding can be of practical importance in the dietary treatment of CRF It is possible that the carbohydrate-rich diet in some patients may produce gastrointestinal complaints because of inadequate digestion of the disaccharides.

Of the dipeptidases ala-pro and gly-leu dipeptidase activities were most depressed in agreement with earlier studies (1 4) This depression of the enzyme activities does not seem to be great enough to interfere with peptide absorption Histochemically Riecken (16) also found depressed enzyme activities (e g. alkaline phosphatase) in some patients with CRF

The dysfunction of the small intestine is also reflected in the increased faecal fat excretion in about 1/3 of the patients low serum folate alues in about half of the patients and generally a flat blood glucose curve after peroral glucose ingestion.

A comparison of the HD group with the uraemic group revealed no difference in respect to morphology and enzyme activities The two NS pa-

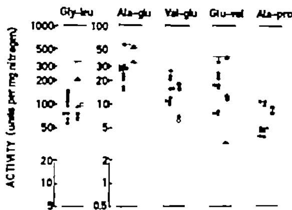


Fig 4 Intestinal dipeptidases in HD (●) uraemic (O) and NS patients (Δ).

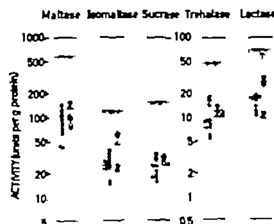


Fig 5 Intestinal disaccharidases in the patients and the reference group Symbols as in Fig. 4

tients had normal morphology and practically normal enzyme activities. Concerning the other renal diseases no characteristic finding was registered However it is of interest to note that both the biopsied patients with HCN (Alport s syndrome) had a distinct increase of plasma cells and elongation of the crypts

In conclusion we have found morphological and functional disturbances of the small intestinal mucosa which can increase our understanding of gastrointestinal complaints occurring in patients with CRF The concepts of uraemic gastritis and colitis are well established Our finding of uraemic enteropathy in most of the patients suggests that the whole gastrointestinal tract suffers in CRF

ADDENDUM

Since acceptance of the present paper McNair and Olsen have published a paper dealing with disaccharidase activity in CRF (Acta med. scand. 195 93 1974). The authors studied the histology nd the lactase sucrase, maltase and alkaline phosphatase activities in jejunal biopsies from 15 patients and performed lactose glucose–galactose and sucrose tolerance tests in 31 patients. They found 2 patients with lactose malabsorption and concluded that no evidence of enzymatic or significant morphological abnormalities was present However the 2 patients with lactose malabsorption had lso decreased activities of sucrase and maltase Moreover 8 biopsies had increased hypercellularity (lymphocytes and plasma cells) and varying degree of oedema.

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TREATMENT OF OSTEOPOROSIS WITH VITAMIN D

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Abstract A daily intake of 35 000 IU vitamin D_3 and calcium supplement for one year could not be demonstrated to influence the bone mineral mass in the forearm of 23 women with osteoporosis.

Andersson et al (1) studied by biopsy technique the incidence of osteomalacia in elderly women They found that morphological evidence of the condition was rare but somewhat more common in women in whom clinical data supported the suspicion of osteomalacia. Hazell and Oatway (6) Exton-Smith et al (5) and Chalmers et al. (4) suggested that osteomalacia caused by vitamin D deficiency is common among the elderly and Smith et al (9) found that the vitamin D activity in plasma was significantly decreased in women with osteoporosis

Vitamin D is a component of many drugs often in combination with calcium. Some of these drugs are recommended for treatment of osteoporosis and although there is no documented evidence of the effect of treatment vitamin D is often given in this condition Some clinicians consider that even in the rich countries there are marginal groups of elderly people with dietary deficiencies Andersson et al. (1) suggest that vitamin D should be introduced as a general prophylactic treatment for the elderly Also the patients frequently seem to benefit from the treatment in that their pain is relieved.

The object of the present study was to evaluate the effect of vitamin D on the bone mass in women with osteoporosis

MATERIAL AND METHODS

Included in the study were 23 women aged 47–78 The criteria for selection were back pain and radiological signs of spinal osteoporosis. All patients had serum creatinine within normal limits T had been operated upon by gastric resection because of peptic ulcer In none of the patients including the two operated upon were there any sign or symptoms of intestinal malabsorption. Iliac crest biopsy with morphometric evaluation of the osteoid tissue (7) revealed no deviation from normal histology except for reduction in size and number of bone trabeculae Serum calcium and serum phosphorus were within normal limits, serum alkaline phosphatase was slightly elevated but not abov what may be expected in women with osteoporosis (7) (Table I) According to our criteria these women must be clinically and morphologically lassified as cases of idiopathic osteoporosis.

All the patients received daily supplement of 35 000 IU vitamin D_3 in water solution and about 1 g calcium in calcium phosphate tablets The treatment was continued for about one year

The bone mineral mas in both forearms was measured at 2–3-month intervals using photon absorption method. The method, very similar to that of Cameron and Sorenson (3), is based on the attenuation of the radiation from an americium-241 source It has previously been described in greater detail (8). Measurements were taken 1 and 6 cm proximally to the distal dorsal edge of the ulna. The average of both arms was used for calculation of bone mass, the two sites are presented separately

Serum calcium, serum phosphorus and serum alkaline phosphatase were measured at 1 week weeks, 1 month 3 6 and 9 months.

RESULTS

Two thirds of the cases were relieved of their back pain during the course of the treatment

There was no significant impro ement of the bone mineral mass in either site of the forearms (Fig. 1)

There were no significant or suggestive ch in the serum calcium serum phosphorus

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Table I. *Serum calcium serum phosphorus and serum alkaline phosphatase*

	Average	S.D	Method	95% confidence limits
Ca/s (mEq/l)	4.87	0.76	Flame photom.	4.5–5.5
P/s (mg/100 cm³)	3.34	0.51	Hurst	..4–4 7
Alk. phosph. (U)	7	1.8	Busch & Busch	–8

alkaline phosphatase during the vitamin D treatment

The two women who had been operated upon because of peptic ulcer could not be demonstrated to deviate from the rest of the group with regard to bone mass No side-effects of the treatment were detected

DISCUSSION

The study was designed to investigate possible effects of a high dose of vitamin D on women classified as osteoporotics and calcium supplement was added to provide at least the daily requirement The dose of vitamin D was approximated to represent the largest amount which can be given without complication to individuals with normal renal function

The findings in this study do not support the hypothesis that osteoporosis can be reversed by vitamin D treatment. The bone mass did not decrease during the observation period. However a loss of bone mineral may not be possible to detect in one year even in women with osteoporosis An extended period of observation will be necessary to investigate possible prophylactic effects of the medication

Finally the remission of symptoms may well be explained as a placebo effect of the intense medical attention experienced by these patients Furthermore the clinical course of spinal osteoporosis is often favourable in that many cases without treatment will in due course be completely relieved of their symptoms until the next episode of vertebral compression

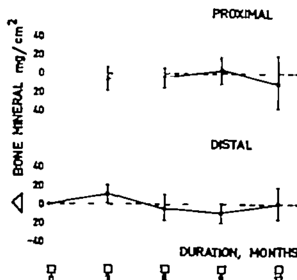


Fig 1 Changes in bone mineral mass in the forearm during vitamin D treatment in 23 women with osteoporosis.

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CLINICAL FINDINGS IN PATIENTS WITH HYPERCALCAEMIA

A Final Investigation Based on Biochemical Screening

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Abstract. Ninety patients with hypercalcaemia (serum calcium 5.6 mEq/l or more) found in the course of 9 months in 1970–71 when a multiphasic procedure was applied for the first time have been reexamined 2 1/2 years later. According to clinical data the patients were divided into six groups: 1) verified primary hyperparathyroidism, 2) probable primary hyperparathyroidism, 3) possible primary hyperparathyroidism, 4) hypercalcaemia of other known cause, 5) hypercalcaemia of unknown cause, and 6) no disturbance of calcium metabolism. The frequency of hyperparathyroidism seems to be higher than in other studies probably because biochemical profiling was applied for the first time to the population in this area. Hyperparathyroidism was the most common cause of hypercalcaemia (28/100 000 inhab./y), closely followed by thiazides (21/100 000 inhab./y) and malignant diseases (16/100 000 inhab./y). Of patients treated with thiazides 0.44% have been found to have hypercalcaemia. When screening procedure is used regularly over a longer period, it seems likely that the most common cause of hypercalcaemia will be found to be thiazides. However from a clinical point of view the diagnosis of hyperparathyroidism remains the main problem.

This report is a follow-up of patients with hypercalcaemia (serum calcium 5.6 mEq/l or more) found from Sept. 1970 to May 1971 (9 months) and presented in this journal in March 1973 (7). This final investigation was made from Feb. to Sept. 1973 about 2 1/2 years after the discovery of hypercalcaemia. In the present paper the final diagnoses are compared to the preliminary ones. The patients' records have been restudied and many patients have been reexamined, especially those with hypercalcaemia of unknown cause. The survey deals with 90 patients with hypercalcaemia (serum calcium 5.6 mEq/l or more). According to the criteria 37 patients had only one high serum calcium value. Two or more high serum calcium values have thus been observed in 64.5% of the total. In a study from Australia (11) the corresponding figure was 51% (77/151) and in a report from Denmark (9) 53.5% (54/101). Of the patients with only one increased serum calcium value 14 have been found to have no disturbance of calcium metabolism.

PATIENTS

The preliminary study (7) dealt with five groups. In the present report patients with no disturbance of calcium metabolism are referred to in a sixth group. Table I shows the number of patients in each group as classified in 1971 and 1973.

Of the 90 patients 25 have been reclassified. In 11 patients the diagnosis of hyperparathyroidism has become more probable or been verified. The previous large group of hypercalcaemia of unknown cause has been reduced after further clinical investigation. The patients are now classified as having no disturbance of calcium metabolism, or the cause of hypercalcaemia has been revealed during the control period. Until now 30 patients (16 ♀, 14 ♂) have died. Nineteen belonged to group 4 (Table I) in which 15 had a diagnosis of malignant disease.

In the original group 5 (Table I) 7 patients have died. Because of the death of five of these patients a further investigation of the hypercalcaemia has not been possible nor has an autopsy revealed a probable cause. Of the original group of 35 patients with hypercalcaemia of unknown cause there now remain only still under observation.

THE SURVEY

Verified primary hyperparathyroidism (n=9: 8 ♀, 1 ♂)

Six patients previously diagnosed as having probable primary hyperparathyroidism have been added to this group. The diagnosis was confirmed by neck exploration in 5 patients and in one at autopsy. The last patient died of heart failure. The histopathological examination

Table I *The six main groups in the survey*
Figures from the 1971 survey within parentheses

	No. of pats.			
	Total	♀	♂	Dead
1 Verified primary hyperparathyroidism	9 (3)	8 (2)	1 (1)	1
2 Probable primary hyperparathyroidism	10 (11)	9 (11)	1 (0)	2
3 Possible primary hyperparathyroidism	7 (6)	4 (5)	3 (1)	2
4 Hypercalcaemia of other known cause*	43 (35)	26 (18)	17 (17)	19
5 Hypercalcaemia of unknown cause	7 (35)	3 (25)	4 (10)	5
6 N disturbance of calcium metabolism	14	11	3	1
Total	90	61	29	30

Including one patient with acute leukaemia and parathyroid adenoma found at autopsy

In these 6 cases showed isolated parathyroid adenomas.

The highest recorded serum calcium level in each of the 9 patients in this group varies from 6.1 to 6.8 mEq/l. Mean age 63 years (range 53–77).

Probable primary hyperparathyroidism (=10 9 ♀ 1 ♂)

Of the original 11 women 5 are still classified as having probable primary hyperparathyroidism and 6 as belonging to group 1. Of the former 5 patients 3 have undergone neck exploration with removal of one or two parathyroid glands with negative histopathological findings. However except for one patient, serum calcium became normal and has remained normal after operation (observation time 1– years). One patient died in 1970 from myocardial infarction but the parathyroid were not examined at the autopsy. The last patient still refuses neck exploration.

Five new patients have now been referred to this group. Two of them were earlier classified as having possible primar hyperparathyroidism. One born in 1884 had an increased serum calcium level from 1967 and died of stroke. Autopsy was not performed. Repeated serum calcium analyses since the last review in 1971 wed constant slight hypercalcaemia. One patient underwent neck exploration with negative findings. *None of th parathyroids was removed and the* serum calcium level is constant at about 6.0 mEq/l. The other 3 patient were earlier classified as having hypercalcaemia of unknown cause. One nderwent a negative neck ploration with partial thyroidectomy after which the serum calcium level decreased and has remained normal for years. No parathyroids were found at the microscopic examination. The other 2 patients are after further clinical and laboratory investigation, classified as having probable primary hyperparathyroidism with only slightly increased level of serum calcium.

The highest recorded serum calcium level in these 10 patients varies from 5.7 to 6.3 mEq/l (most above 6.0 mEq/l). Mean age 68 years (range 40–89).

Possible primary hyperparathyroidism (=7 4 ♀ 3 ♂)

In the original investigation 6 patients belonged to this group. Two of them have now been classified as having probable primary hyperparathyroidism as noted above. Two are still regarded as having possible primary hyperparathyroidism. The other 2 have now been referred to group 4 hypercalcaemia of known cause other than primary hyperparathyroidism. In one of these it is likely that the hypercalcaemia is due to a thiazide. The other patient was very old and bedridden. She died of myocardial infarction. Autopsy revealed normal parathyroids and osteoporosis. The slight hypercalcaemia may have been caused by the immobilization.

Five new patients have now been referred to this group. One was earlier diagnosed as thiazide-induced hypercalcaemia (group 4). The follow-up 2 years after withdrawal of the thiazide shows a constant slight hypercalcaemia. Three patients formerly in group 5 hypercalcaemia of unknown cause have constant hypercalcaemia without obvious evidence of other diseases. These patients are 79–83 years of age and have not been investigated further. In the fifth patient, also formerly in group 5 the serum calcium level has gradually decreased and is now just below the upper normal limit. Other investigations, such as X-ray of bones, lungs and kidneys, renal calcium excretion, all th negative findings and an observation time of three years, make the diagnosis of hyperparathyroidism probable.

Highest recorded serum calcium level in these patients varies from 5.6 to 6.6 mEq/l (most below 6.0 mEq/l). The mean age is 72 years (range 51–83).

We believe that all 26 patients in groups 1–3 have hyperparathyroidism. However in group 3 it has not been possible to confirm the diagnosis because of the patients high ages and the fact that some have died during the course of the investigation. Since a follow-up of nearly three years has shown persistent hypercalcaemia (except 1 one case) and absence of other diseases causing hypercalcaemia, we believe that the assumption i justified that also the patients in group 3 have hyperparathyroidism.

Hypercalcaemia of other known cause (=43 26 ♀ 17 ♂)

This group earlier comprised 35 patients of whom only one has been reclassified. This patient was believed to have thiazide-induced hypercalcaemia but i now referred to group 3 as mentioned above.

Table II *Hypercalcaemia of other known cause than primary hyperparathyroidism*

Figures from the 1971 survey within parentheses if the number of patients has changed

	No of pats.			
	Total	♀	♂	Dead
Thiazides	20 (14)	16 (10)	4	2
Calcium (p. o.)+thiazide	2	1	1	1
Calcium (i. v.)	1	1		
Calcium-vitamin D (renal transpl.)*	1		1	
Ergocalciferol	1		1	
Malignancies incl. haematological malignancies	15 (14)	6 (5)	9	15
Thyrotoxicosis	2	1	1	
Immobilization	1	1		1
Total	43 (35)	26 (18)	17 (17)	19

Persistent hypercalcaemia for 3 years and now classified as slight tertiary hyperparathyroidism.
Including one patient with acute leukaemia and parathyroid adenoma found at autopsy

Nine patients formerly with other diagnoses have now been referred to group 4. Two patients from group 3 are classified as having thiazide-induced hypercalcaemia and hypercalcaemia caused by immobilization respectively (see above). Out of 7 patients from group 5 the cause of hypercalcaemia in 6 was found to be thiazides. The remaining patient was operated upon for hypernephroma without metastases. After nephrectomy serum calcium remained normal. She died of pulmonary embolism one year after operation.

Table II shows the different causes of hypercalcaemia in group 4 and the number of patients in each subgroup.

Hypercalcaemia of unknown cause (n=7 3 ♀ 4 ♂)

In this group which originally contained 35 patients, there now remain only 7. Five of these patients died before the final study in 1973. Autopsy was performed on only one patient who had an uncomplicated cancer of the stomach and final unexplained increase in serum calcium as demonstrated in Fig. 14 in the preceding paper (7). One patient underwent negative parathyroid exploration with no change of serum calcium level after operation. The remaining patient has a slight sporadic hypercalcaemia, as was also found in 4 of the afore mentioned 5 patients.

In 14 of 35 patients the diagnoses are now more accurate, 3 are classified as having probable primary hyperparathyroidism (group 2) and 4 as having possible primary hyperparathyroidism (group 3). Seven patients have been referred to group 4. The cause of the hypercalcaemia in 6 of the latter was found to be thiazides. The last patient was operated upon for hypernephroma. All these 14 patients have been mentioned previously.

No disturbance of calcium metabolism (n 14 11 ♀ 3 ♂)

In 14 cases of the 35 in group 5 further investigation has not revealed any disturbance of calcium metabolism. Each of these 14 patients had only one slightly increased serum calcium value. They are now referred to group 6, no disturbance of calcium metabolism.

DISCUSSION

When judging the patients with hypercalcaemia in this material it should be remembered that serum calcium analyses have been part of a biochemical profile which has been performed on in- and out-patients in this area, even if only one or two of the tests have been requested by the physician. The number of serum calcium analyses performed each year is more than 70 000 in a population of nearly 1.5 000. This figure is, of course, much higher than in other areas where similar investigations of the cause of hypercalcaemia have been made. However, the actual number of patients investigated cannot be determined at present.

In the preliminary study (7) the classification of patients was done within one year of the discovery of hypercalcaemia. The present study was performed 1 / years later and mainly based on routine procedure, which means that there has been no special programme for intensive follow-up. The patients are also rather elderly and have only moderately high serum calcium levels. This may explain why it has taken so long to establish a final diagnosis.

Hyperparathyroidism

The number of patients with primary hyperparathyroidism (verified, probable and possible) is 28/100 000 inhab./y. Even if group 3, possible hyperparathyroidism, is excluded, the figure is still very

high 20/100 000 3–4 times higher than the usual number found in this area (7) and reported in other surveys (8 10 12 17) This may of course be due to the fact that these patients have been found when a multiphasic procedure is applied for the first time Since the mean age in this material is higher than in other surveys (3 5 9 13) it seems likely that the patients may have had their hyperparathyroidism for many years The sex distribution (21 ♀/5 ♂) differs from the general report of 2/1 (13)

Malignant diseases

The incidence of hypercalcaemia caused by malignant diseases including haematological malignancies is 16/100 000 inhab./y In the preliminary study (7) the impression was that many patients with malignant diseases had hypercalcaemia without metastases However most patients developed metastases rather soon after the previous survey and died In one patient with hypernephroma (Fig. 12 (7)) serum calcium returned to normal after nephrectomy but increased when metastases appeared. In another patient with hypernephroma serum calcium remained normal until death 1 year after nephrectomy All 15 patients with malignancies have died within 1 / year This confirms the bad prognosis of malignant disease complicated by hypercalcaemia (18) Of all patients in this area with carcinoma of the lung, kidney breast, oesophagus and stomach and leukemia and multiple myeloma, 16% had hypercalcaemia. A detailed analysis of the frequency of hypercalcaemia in the different malignancies is not possible since the material is too small The overall sex distribution is 6 ♀/9 ♂

Thiazides

The incidence of thiazide-induced hypercalcaemia is 1/100 000 inhab /y Since a continuous registration of prescriptions in this area started in 1968 (1) it has been possible to calculate the frequency of hypercalcaemia in patients treated with thiazides During the period of this investigation the commonly used thiazides (bendroflumethiazide chlorthalidone clopamide and polythiazide) were prescribed to 4740 patients/100 000 inhab /y The frequency of thiazide induced hypercalcaemia found therefore, is approximately 0 44% However in 1970–71 when this material was collected analyses were seldom performed on patients outside the hospital The true incidence of hypercalcaemia caused by thiazides may therefore be several times higher than 0.44% A more thorough investigation of this problem will be possible in this region in the near future The sex ratio in this material is 16 ♀/4 ♂

As a rule the hypercalcaemia caused by thiazides is mild. Constant hypercalcaemia with levels above 6 0 mEq/l seems according to this and other investigations (4 16) to be unusual

Sarcoidosis

In this study no patient with sarcoidosis was found The number of patients with sarcoidosis investigated with serum calcium determinations at this hospital in 1970–71 was 58 None of them had hypercalcaemia. This may be compared to a previous study in this area in 1940–56 (15) when serum calcium determinations were performed on 185 patients with sarcoidosis of whom 43 (23.2%) had hypercalcaemia (serum calcium level above 11 0 mg/100 ml) Hypercalcaemia caused by sarcoidosis seems nowadays to be more rarely observed as has also been reported by others (6)

No disturbance of calcium metabolism

In 14 patients the initially recorded isolated high serum calcium level has not been confirmed nor has further investigation revealed signs of disturbed calcium metabolism In other materials (9 11) this figure seems to be higher This may be due to the fact that our patients have been followed up for a rather long time and that the upper normal limit used in this survey may be slightly higher than in other studies. The obvious reason for isolated high calcium levels in patients without disturbed calcium metabolism is technical errors They may be due to analytical errors erroneous handling of the blood samples or faulty techniques when drawing blood (2 14) However these errors also affect other analyses in the battery of tests used Since this does not seem to be the case in the present patients technical errors do not seem to be a major explanation of the high calcium levels found in this group

CONCLUSION

This final investigation suggests that hyperparathyroidism is the most common cause of hypercalcaemia, closely followed by thiazides and

malignant diseases. However many patients with hyperparathyroidism are rather elderly and have only slight clinical symptoms which require a long period of observation in order to obtain a final diagnosis. It seems likely that a further use of screening procedure will show that thiazides are the most common cause of hypercalcaemia. From a clinical point of view the diagnosis of hyperparathyroidism remains the main problem

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BLOOD TRANSFUSION REQUIREMENTS BEFORE AND AFTER BILATERAL NEPHRECTOMY IN PATIENTS UNDERGOING CHRONIC HEMODIALYSIS

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Abstract. Blood transfusion requirements have been calculated in 6 patients undergoing chronic hemodialysis before and after bilateral nephrectomy. Following nephrectomy the monthly average transfusion requirement per patient increased from 379±135 to 673±312 ml (±S.D.) ($p<0.03$) and average Hb decreased from 7.7±0.4 to 6.9±0.5 g/100 ml ($p<0.03$). Furthermore transfusion requirements were calculated in 10 nephrectomized and 15 non-nephrectomized patients. The monthly transfusion requirement averaged 404±181 ml per patient in the nephrectomized against 14±22 in the non-nephrectomized group ($p<0.01$). Hb averaged 5.3±0.7 g/100 ml in the nephrectomized against 6.7±1.– in the non-nephrectomized group ($p<0.01$). No significant difference in age, body weight, length of observation period, average predialytic serum creatinine, average predialytic BP or duration of antibiotic therapy was found in the two groups. Seven of 9 multitransfused patients developed lymphocytotoxic antibodies. It is concluded that severely atrophic kidneys exert a significant, although limited, erythropoietic stimulating function and that nephrectomy should not be performed as a routine in patients on chronic dialysis awaiting renal transplantation.

Uremia is almost invariably accompanied by anemia which is normochromic and normocytic (3) and there appears to be a rough proportionality between the severity of uremic intoxication and the degree of anemia (4). Possible contributing factors to this anemia are increased hemolysis (1 3 4 5 6, 7 8 13) depression of marrow function due to accumulation of uremic toxins (3 4 11 12) and inadequate production of renal erythropoietic stimulating factor (REF) (4). It is now presumed by many investigators that failing production of REF plays a major role in the development of uremic anemia (3 4 5 6 8) inasmuch as regression of the uremic condition by dialysis brings only a minor impro ement of anemia and rate of erythropoiesis (5 6 8) and hemolysis in most cases is slight and should be ... ly compensated for if the marrow erythrop... tic function were normal (3).

It is clearly established that the kidney ... mal man is the main organ for production of F... (4) although extrarenal production ha been de... onstrated (9 10).

It has been suggested that se erely atr... kidneys are unable to stimulate erythropoies... 1.. 13). The observation that transfusion equi... ments seemed to be higher in nephrect... mized th... in non-nephrectomized patients undergoing ... hronic hemodialysis prompted the present stud... The aim was to evaluate whether severely atroph... kidneys without excretory function had any dete... table influence on erythropoiesis by comparing transfusion requirements in the two mentioned groups of patients.

PATIENTS AND METHODS

The material comprises 31 patients (17 males, 14 females) undergoing chronic hemodialysis within the period June 1967 – Oct. 1973 having prenephrectomy observation periods ≥6 month and postnephrectomy observation periods ≥9 months, and not showing signs of severe hemolysis judged by reticulocyte counts, serum bilirubin and Coombs' test. The patients were di...ded into three groups.

Group I. Six patients observed both immediately *before and after* nephrectomy within the period June 1967 – June 1971 (Table I). Transfusions given during and in the first 3 month following nephrectomy were not included in the study. In this part of the investigation period the transfusion ...] at stabiliz... ing the Hb level ... g/1(... ...sion were administered ... ...d. All pa-

Table I. *Average transfusion requirements and Hb concentrations (±S D) before and after bilateral nephrectomy in group I*

Sex	Age (y)	Transfusion (ml/mo) Before	After	Hb (g/100 ml) Before	After	Diagnosis
9 ♀ 4 ♂	38 ±5 7	379±135 p<0 03	673±202	7 7±0 4 p<0.03	6 9±0.5	1 chronic glomerulonephritis 3 chronic pyelonephritis 2 polycystic kidneys

tients were nephrectomized in preparation for renal transplantation. Patients in group I served as their own control before and after nephrectomy

Group II Ten patients observed *after* nephrectomy within the period July 1971 Oct. 1973 (Table II) In this part of the investigation period the transfusion policy had changed Hb was allowed to drop and transfusions were restricted to patients with pronounced anemic symptoms. Four patients were nephrectomized on the indication of severe hypertension 6 in preparation for renal transplantation

Group III Fifteen patients retaining their kidneys (Table II) observed in the same period as patients in group II.

Dialysis was performed for 10 hours twice weekly using Gambro-Alwall (R), Gambro-Lundia (R) or B-D (Dewids, personal communication) kidneys except for a few patients in group I in whom double-layer Kiil kidneys were used

All transfusions were administered as packed RBC The patient received diet containing an average of at least 0.86 g protein/kg b wt /day together with adequate supplies of vitamin Some patients in group II and most in group III received iron orally

All patients had creatinine clearance below 1 ml/min many were anuric None suffered from excessive ... or gastrointestinal blood loss during the observation period all were normotensive without receiving antihypertensive therapy Hb serum creatinine serum urea and BP were recorded at the beginning of each dialysis in group I patients and before dialysis once every month in group II and group III patients and the mean values were calculated

Since infection is known to depress erythropoiesis, the per cent duration of antibiotic therapy during the observation period was assessed in each patient.

RESULTS

Group I After nephrectomy transfusions had to be increased from an average of 379±135 ml/month (±S D) to 673±202 in an attempt to maintain prenephrectomy Hb levels (Table I and Fig. 1) Judged by the Wilcoxon test for paired values the *difference is significant* (p<0 03) *In spite of the* increase in transfusions Hb dropped from 7 7±0 4 g/100 ml to 6 9±0.5 (p<0 03)

There was no significant difference between the duration of pre and postnephrectomy observation periods BP serum creatinine serum urea or duration of antibiotic therapy (Table III)

Groups II and III The transfusion requirement averaged 408±181 ml/month in the nephrectomized (Table II) against 14±22 in the non-nephrectomized group (*Table II* and Fig. 2) Judged by the Mann-Whitney test for unpaired values the difference is significant (p<0 01) Hb averaged 5 3±0 7 g/100 ml in the nephrectomized (Table II) against 6 7±1 2 in the non-nephrectomized group (p<0 01) No significant difference between age body weight, length of observation period, serum crea-

Table II *Average transfusion requirements and Hb concentrations (±S.D) in groups II and III*

	Sex	Age (y)	B wt (kg)	Transfusion (ml/mo)	Hb (g/100 ml)	Lymphocytotoxic antibodies	Diagnosis
Group II (n=10)	9 ♀ 1 ♂	39 7±8 3	55 5±11 4	404±181	5 3±0 7	7	3 chronic glomerulonephritis chronic pyelonephritis 3 nephrosclerosis polycystic kidneys
Group III (n=15)	3 ♀ 1 ♂	38 7±13.8	6 7±10 5	14±22 p<0 01	6 7±1.2 p<0.01	0 p<0.01	10 chronic glomerulonephritis chronic pyelonephritis 2 nephrosclerosis 1 polycystic kidneys

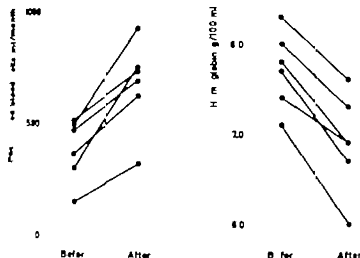


Fig. 1. Transfusion requirements and Hb concentration (±S.D.) before and after bilateral nephrectomy in group I

twine, BP or duration of antibiotic therapy was found in the two groups (Tables II and IV)

Serum urea was significantly higher in group II and lymphocytotoxic antibodies were present in 7 patients in group II and in none of the patients in group III (Tables II and IV)

DISCUSSION

The present data indicate that nephrectomized dialysis patients require more transfusions than non-nephrectomized in order to maintain the same Hb level. The difference in transfusion requirements does not seem to be caused by differences in uremic intoxication, BP, frequency of infections, body weight or nutritional state.

Patients in group II had undergone chronic dialysis for a significantly longer period than patients in group III (Table IV), a factor known to reduce transfusion requirements and improve erythropoiesis (5, 6, 8).

Group II has an overweight of females, but none of these suffered from menstrual blood loss which could be responsible for the higher transfusion requirements. Most of the patients in this group initiated dialysis treatment when transfusion policy was liberal and, in spite of a later very restrictive policy, only a slight reduction in transfusion requirement was obtained except in one patient.

The observed difference in serum urea between patients in groups II and III is caused by dietary indiscretions and does not seem to be of such order of magnitude as to be responsible for the difference in transfusion requirement (4).

It is clearly established that the anemia of uremia is at least in part, caused by insufficient production of REF (3, 4, 5, 6, 8, 10). The question is whether severely atrophic kidneys as found in patients undergoing chronic dialysis treatment, are still capable of stimulating erythropoiesis. Nathan et al. (11, 12, 13) found that erythropoiesis was unchanged in 7 patients after nephrectomy and concluded that severely atrophic kidneys seemed to be erythropoietically inactive. This view has recently been opposed by other workers (1, 7, 15).

Van Ypersele de Strihou and Stragier (15) found

Table III. *Clinical and laboratory data before and after bilateral nephrectomy in group I*

Observation period (mo.)		Predialytic serum creatinine (mg/100 ml)		Predialytic serum urea (mg/100 ml)		Predialytic BP (mmHg)	
Before	After	Before	After	Before	After	Before	After
12.7±1.6	11 ±3.3	1 3± 7	1. 5± 1	177±18	143±18	138/88	123/76

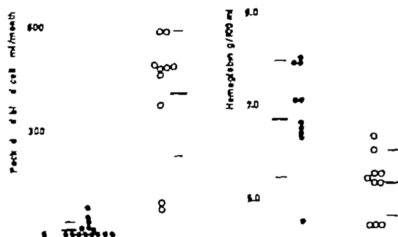


Fig 2 Transfusion requirements and Hb concentrations (±S D) in groups II (O) and III (●).

significantly higher transfusion requirements in nephrectomized than in non-nephrectomized patients The difference was equally noticeable in males and females Blumberg and Keller (1) observed an impressive drop in hematocrit following nephrectomy in 8 patients (7 males 1 female) undergoing chronic dialysis treatment. The female patient became dependent on regular transfusions after nephrectomy

Kominami et al (7) noted that nephrectomy consistently lowered h matocrit augmented the transfusion requirement and decreased the rate of erythropoiesis u judged from changes in plasma iron turnover and erythro d iron turnover Half of the patients quiring transfusions before neph- ha t o e t nsfused regularly after neph- *my eph tomy did not cause any change* al time Naets and Wittek (10) demtrated that nephrectomy significantly reduced the number of medullary normoblasts in uremic patients.

From these data and the present study it appears likely that failing, atrophic kidneys without excretory function are able to exert erythropoietic stimulating activity probably through a remaining, albeit limited, endocrine function.

Differences in the production rate of extrarenal erythropoietic stimulating factor (9 10) could be the explanation of the variable transfusion requirements observed in anephric patients.

The disadvantages of transfusions include the costs the risk of transfusion reactions the risk of transmitting viral hepatitis which is a major problem in many dialysis units (14) and the risk of sensitizing candidates for renal transplantation to transplantation antigens as demonstrated by the presence of lymphocytotoxic antibodies in 7 of 9 multitransfused patients (Table II) In consequence of this a relation between the number of *pretransplantation transfusions and the frequency* of graft rejection has been clearly demonstrated (2)

The conclusion from the presented data is that nephrectomy should be avoided as a routine procedure prior to renal transplantation and should be performed only on strict indications

Table IV *Data of patients in groups II and III*

	Dialysis before study (mo)	Observation period (mo)	Antibiotic treatment (% of days)	Predialytic serum creatinine (mg/100 ml)	Predialytic serum urea (mg/100 ml)	Predialytic BP (mm Hg)
Group II ($n=10$)	17 7±7 7	19 6±7 0	2.1±3.3	12.2±1 7	172±18	132±16/77±9
Group III ($n=15$)	2.9±4 4 $p<0.01$	15.5±7 6	2.9±5.3	13 6±3.3	141±36 $p<0.01$	137±9/77±8

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THE EFFECT OF EXTRACORPOREAL IRRADIATION OF THE BLOOD IN NECROKIDNEY TRANSPLANTATION

Three Years Follow-up Study

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Abstract Sixty-nine patients received xtracorporeal irradiation of the blood (ECIB) as pretreatment and as regular follow-up treatment in connection with necrokidney transplantation. Seventy-four patients did not receive ECIB. Both groups were treated with azathioprine and prednisone according to conventional standards The results were evaluated by the cumulative fraction of patients without rejection, the cumulative graft survival and the graft function An intergroup analysis showed that the ECIB-treated patients had significantly fewer rejection episodes ($p<0.001$), whereas a 10% improvement in graft survival was insignificant ($p>0.10$) and graft function dentical. An intragroup analysis showed a 25% improvement in graft survival after 1 year in 26 patients who had received sufficient treatment with ECIB as compared to 27 insufficiently treated patients and to the untreated group. Also this result was, however not significant in the small groups under study Despite well documented quantitative and qualitative effect on human lymphocytes, ECIB as it can be administered in practice on large scale is of little if any value in necrokidney transplantation. On the other hand the results do not permit the conclusion that ECIB is entirely ineffective in humans, and certain possibilities for improvement of the method are discussed

In the present paper late results of necrokidney transplantations in patients undergoing extracorporeal irradiation of the blood (ECIB) before and after grafting are presented

MATERIAL AND METHODS

General description of the material

Patients In the period Dec. 10, 1968 – March 1 1973 total of 169 necrokidney transplantations were carried out at Rigshospitalet. Nineteen of these transplantations ere retransplantation and are excluded because analysis of larger materials indicates that second graft h ve poorer prognosis (11) and because the present material is too small to permit separate analysis of the effect of ECIB in retransplantation Among the remaining 150 consecutive patients with first necrokidney grafts 7 are excluded for the following reason T patients received ECIB at another center ere retransferred to this center shortly after transplantation and have thus not been followed here Four patients ceived ECIB only as a follow-up treatment starting immediately after transplantation This small material does not permit separate evaluation of this type of ECIB schedule The last patient was non-ECIB-treated 4-year-old child who died from rejection 3 weeks following grafting This case was excluded as being the only child in the material.

The remaining 143 patients with first necrokidney grafts comprise 91 patients, whose course until Oct. 1 1971 has been described in previous paper (15) and who have since then been followed for further 17 months, as well as 52 new patients who ha been transplanted between Oct. 1 1971 and March 1 1973 The mean age of the 143 patients, 65 men and 78 women was 42 years (range 14–59) at the time of grafting

The diagnosis of the primary kidney disease was established on conventional clinical criteria, supplemented by histological examination of renal biopsies and/or of kidneys removed at total nephrectomy With du reservation for the well know fact that an exact histological diagnosis is not alw ys possible in maximally contracted kidneys (end stage kidneys") the primary kidney disease was classified as glomerulonephritis in 57 as chronic interstitial nephropathy ("pyelonephritis) in 63 and as various other kidney diseases, primarily polycystic kidney disease in 23 patients.

The donor kidneys were all necroorgans, 135 from Scandiatransplant and 8 from other regions. The mean age of the donors, 90 men and 53 women, was 35 years (range 10–64).

The ischemic periods of the donor kidneys wer fined in the conventional manner (15). The m

ischemic period was 19 min (range 0–50) and the mean cold ischemic period 7.5 hours (range /₂–22).

The tissue typing was carried out by the Tissue Typing Laboratory at Rigshospitalet using the method of Kissmeyer-Nielsen and Thorsby (7). The results are graded as A, C D and E matches according to conventional criteria (8) and are presented as worst possible match i.e. non-identified antigen(s) are considered incompatible. Among the 143 patients there were 11 A 59 C 70 D and 3 E matches. In our limited material there was no significant correlation between match grade and the result of necrokidney transplantation. Nevertheless histocompatibility testing is still carried out, and the results are presented in Table II.

Lymphocytotoxic antibodies The presence of lymphocytotoxic antibodie was examined monthly in all patients prior to grafting. In 28 of the 143 patients non-specific lymphocytotoxic antibodies could be demonstrated at some interval before transplantation. In 4 of these patients antibodies active against the donor's HL-A antigen(s) were demonstrat d, but only in one were they found at the time of transplantation (F match).

The transplantation were performed with conventional technique A ureter bladder anastomosis was used in almost all cases.

Bilateral nephrectomy was carried out before transplantation in 17 patients in connection with transplantation in 58 and after transplantation in 10 Thus 85 (59%) of the 143 patient were totally nephrectomized, wherea 58 (41%) were not nephrectomized.

The immunosuppressive therapy after transplantation consisted of azathioprine (Imurel®) and of prednisone The dose of Imurel was kept constant throughout and ...ged 1.6 mg/kg b wt. The dose of prednisone was ...ally about 1–3 mg/kg b wt. It was gradually de...sed over 5 weeks to 0.5 mg/kg, and during the fol...wing year to 0.3 mg/kg. After 2–3 years the average dose was 0.1–0.2 mg/kg Twenty-three patients received local graft irradiation with 150 rads 3 times during the first week after transplantation

Treatment of rejection crise Rejection crises were treated with high doses of prednisone or prednisolone parenterally or perorally (0.5–2.0 g/day) and heparin in a dose of 50 mg. 4 times/24 h Seven non-ECIB-pretreated patients moreover eceived ECIB during rejection.

ECIB treatment

Sixty-nine (48%) of the 143 patients received ECIB before transplantation and as a egular follow-up treatment, starting on the first or second postoperative day (group I). Seventy-four (52%) of the 143 patients received ECIB neither as pretreatment nor as regular follow-up treatment after transplantation (group II). Seven of the patients in group II did however receive ECIB in addition to an increase of prednisone and administration of heparin during actual ejection crises.

ECIB and non-ECIB-treated patients were transplanted over the entire observation period Dec. 16, 1968 – March 1 1973

The irradiators were constructed by the Danish Atomic Energy Commission, Research Establishment Risø Denmark, and have been described in detail elsewhere (13). Fifteen of the 69 ECIB-treated patients received ECIB in a stationary gamma unit (ECIB I), 5? were treated in a mobile gamma unit (ECIB II), and 2 in a portable beta unit (ECIB III)

Definition Transit dose (TD) is the radiation dose received by an item while in transit through the radiation field. The *number of blood volumes radiated (N BV rad.)* is calculated from the measured blood flow rate and the blood volume of the individual patients. The total irradiation dose *mean cumulated erythrocyte dose (MCED)* is the product of TD and No BV rad. but is of limited meaning as far as lymphocytes are concerned since these cells are not restricted to the blood stream.

ECIB schedule In group I the schedule recommended by Cronkite and Chanana (3) was used i.e. pretreatment before transplantation and follow-up treatment after transplantation.

Follow-up treatment could easily be adjusted to follow the same principles as in the experimental situation or in the clinical situation with live donor grafts, since it could be commenced on the first or second day after grafting. Treatment lasted on an average for 1 days In the present material the mean TD was 300 rads (range 44–550), the mean No BV rad. 115 (range 52 318) the mean MCED 40200 rads (range 1600–88300) and the mean duration of treatment 60 hours (range 3–183) There was no significant difference in the follow-up treatment between patients receiving different types of pretreatment.

Pretreatment should ideally be completed as shortly before grafting as possible This can easily be done in the experimental situation and in the clinical situation, when live donors are used Such as conditions are today with respect to availability of donor organs, the same strict program is practically impossible in necrokidney transplantation. Sometimes the pretreatment is not completed when a kidney becomes vailable. In other instances a variable interval has elapsed between completion of pretreatment and grafting Furthermore, in our material a study was made on the effect of varying TDs on the rate of development and duration of lymphopenia (14). As a result of all the mentioned factors pretreatment in the present material became less uniform than follow-up treatment. The data are the following: mean TD 300 rads (range 14–650) mean No. BV rad. 170 (range 8–482), mean MCED 53000 rads (range 300–85200), mean duration of treatment 87 hours (range 5–151) and mean interval between cessation of pretreatment and grafting 5 months (range 1 d. 18 mo). Among the 69 patients with the above mentioned average values for pretreatment 16 received a mean TD of 100 rads and treatment was in all cases completed before transplantation. Fifty-three ... received mean of 300 rads and tr... completed, whereas 8 wer grafted befor... of treatment.

Table I. *Clinical data of the 143 patients (mean ± 1 S.D.)*

No. of pats	Age (y)	Sex	Period between grafting and follow-up (mo)	Primary kidney disease: Glomerulonephritis	Primary kidney disease: Other	Duration of dialysis (mo)	Not dialysed	No. of pats who have received 20 U blood before transplantation	No. of pats with lymphocytotoxic antibodies
Group I [illegible]	42 ±11	32 ♂ 37 ♀	1 0 ±1..5	33	36	13 ±12	6	35	21
Group II 74	43 ±11	33 ♂ 41 ♀	4 4 ±11.8	4	50	9 7 ±9.8	15	19	7
Difference	n.s.	s.	n.s	n.s.		n.s.	n.s.	$p<0\ 01$	$p<0\ 01$

In order to elucidate whether variations in pretreatment might influence the clinical results the following subgroups were selected within group I

High TD and low TD. Comparison could be made between two subgroups with the following data. *High TD* 45 patients were given a complete series of ECIB with the following mean values. TD 300 rads (range 260-650) No BV rad 170 (range 75–303), MCED 53 500 rads (range 32 400–85 200) and duration of treatment 87 hours (range 41–151). *Low TD* 16 patients were given a complete series of ECIB with the following mean values. TD 100 rads (range 14–120) No. BV rad 195 (range 36–139), MCED 19 000 rads (range 6 700–40 000), and duration of treatment 76 hours (range 36–139).

Sufficient and insufficient ECIB Among 53 patients who had received mean dose of 300 TD two subgroups were defined on the basis of previously published (14) results on the degree and duration of lymphopenia following different ECIB schedules *Sufficient ECIB* 26 patients had more than 75 No. BV rad and an interval between cessation of ECIB and grafting of less than 5 months. *Insufficient ECIB* 27 patients had less than 75 No. BV rad and/or an interval between cessation of ECIB and grafting of more than 5 months.

Evaluation of the esults

The clinical results were evaluated from the cumulative fraction of patients without rejection episodes within the whole period of observation the cumulative graft survival (until failure of graft or death from all cause) and the graft function 12, 4 and 36 months after transplantation.

Rejection episodes Only acute rejection episodes considered, since chronic rejection in the form of slowly deteriorating renal function will ppear from the analysis of graft survival and graft function as stated below

Acute rejection episodes are here defined as significant reduction in endogenous creatinine clearance of functioning graft in at least two consecutive determinations and after exclusion of other causes of acute post transplant renal failure by isotope nephrography l. rography (or retrograde pyelography) graft angiography and, if deemed necessary graft biopsy. In grafts with delayed onset of function the diagnosis of rejection was much more difficult and frequently required explorative surgery (with biopsy) to exclude other complications Since delayed onset of function (and never functioning grafts) was equally distributed in groups I and II (Table II) however this special prob-

Table II *Data of the 143 donors (mean ± 1 S.D.)*

No. of donors	Age (y)	Sex	Ischemic periods: Warm (min)	Ischemic periods: Cold (h)	Initial graft function: Immediate	Initial graft function: Delayed	Initial graft function: Never functioned	Match grades: A	Match grades: C	Match grades: D	Match grades: E
Group I 69	37 ±15	44 ♂ 25 ♀	19 6 ±16.1	8 0 ±4 6	40	1	8	4	23	40	2
Group II 74	33 ±16	46 ♂ 28 ♀	19 0 ±13.3	7 4 ±4 1	44	21	9	7	36	30	1
Difference	s.	n.s	n.	n.	s.		n.s.		n.s.	s.	n.s.

Acta

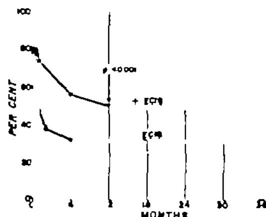


Fig. 1. Cumulative fraction of patients without rejection episodes for the ECIB and the non-ECIB-treated groups.

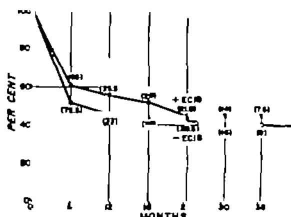


Fig. 2. Cumulative graft survival for the ECIB and the non-ECIB-treated groups. Figures within parentheses indicate no. of patients at risk.

lem, which is restricted to the immediate posttransplant period hardly invalidates the estimation of a possible difference in the frequency of acute rejection episodes in the two groups.

With regard to *graft survival* no attempt was made to distinguish between "immunological" and "non-immunological failures".

The graft function was estimated by the 24-hour endogenous creatinine clearance/1.73 m² BSA by the presence of hypertension and by the presence of nephrotic type proteinuria (>2.5 g/24 h).

Statistical methods were life table analysis, Fisher's exact probability test, χ^2-test, Student's *t*-test.

An intergroup analysis was made between groups I and II.

An intragroup analysis was made within group I comparing high and low TDs and sufficient and insufficient ECIB as defined above.

RESULTS

Comparison of results in groups I and II

The clinical data of the recipients, the donors and the immunosuppressive therapy after transplantation are presented in Tables I, II and III for the ECIB-pretreated group I and the non-ECIB-pretreated group II. The two groups were comparable considering the presented data except for the number of patients with non-specific lymphocytotoxic antibodies and the number of blood transfusions before transplantation, which was greater in group I ($p<0.01$) and the number of patients who received local graft irradiation after grafting, which was greater in group II ($p<0.01$).

Fig. 1 shows the cumulative fraction of patients in groups I and II presenting no rejection episodes within the whole observation period of 42 months. The difference between the two groups is significant ($p<0.001$).

Fig. 2 presents the cumulative graft survival for groups I and II. The fraction surviving after 1 months was 10% higher in group I, but this difference is not significant ($p>0.10$).

Fig. 3 shows the function of the grafted kidneys in groups I and II 12, 24 and 36 months following transplantation. The difference between the two groups is not significant ($p>0.1$).

A comparison between groups I and II excluding 80 patients (49 from group I and 31 from group II) who 1) had lymphocytotoxic antibodies, and/or had received 2) more than 20 U blood prior to grafting, 3) local graft irradiation and 4) had received an insufficient ECIB treatment

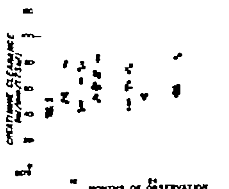


Fig. 3. Creatinine clearance (median of 3 observations) at 12, 24 and 36 months following transplantation for the ECIB and the non-ECIB-treated groups. O = patients with normal BP. ● = with elevated BP treated with antihypertensive therapy. × = with nephrotic syndrome.

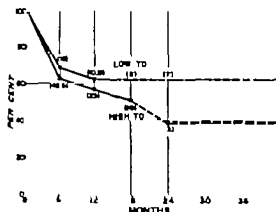


Fig. 4. Cumulative graft survival within the ECIB-treated group receiving high and low TDs as pretreatment. Figures within parentheses indicate no. of patients at risk.

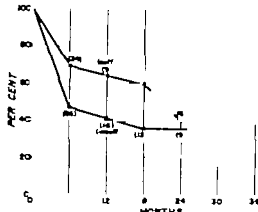


Fig. 5. Cumulative graft survival within the ECIB-treated group receiving sufficient and insufficient pretreatment. Figures within parentheses indicate no. of patients at risk.

or ECIB only during rejection episodes did not reveal any significant difference from the results presented in Figs. 1, 2 and 3.

Comparison of results with high and low TDs

Fig. 4 shows that there is no significant difference in the cumulative graft survival in 45 patients receiving a complete ECIB treatment with a high TD (300 rads) and 16 patients receiving a complete ECIB treatment with a low TD (100 rads).

Comparison of results after sufficient and insufficient ECIB

Fig. 5 shows the cumulative graft survival in 26 patients receiving a sufficient and 27 receiving an insufficient ECIB treatment. It can be seen that patients receiving an insufficient ECIB treatment present a graft survival almost identical to that of the untreated group II (cf. Fig. 1). It also appears that the sufficiently treated patients have a 25% better graft survival after one year than the insufficiently treated. The difference is, however, not statistically significant ($p>0.1$).

Comparison of infectious complications in groups I and II

Table IV shows that severe infectious complications were of equal frequency in the ECIB-treated and the non-ECIB-treated groups. With the exception of herpes zoster, by far the majority of infectious complications occurred within the first 3 months after grafting.

DISCUSSION

The immunosuppressive effect of ECIB upon skin and kidney graft survival has been investigated in experimental studies (1, 3, 6, 1). In goats pregraft ECIB according to the conventional schedule with 3–10 BV radiated/day at a TD of 700–100 rads, resulted in kidney graft survival of 14–4 days which was not significantly longer than the graft survival in untreated goats (15–19 days). In contradistinction pregraft ECIB with many BV radiated/day in order to irradiate a larger fraction of the recirculating pool of lymphocytes resulted in a significant prolongation of the graft survival (21–59 days). In two goats from this group pre and postgraft ECIB resulted in graft survival of 29 and 84 days (1).

Based upon these experimental results Cronkite and Chanana (3) have proposed the following ECIB schedule: 1) prior to grafting to deplete the body of lymphocytes, 2) following grafting to

Table III. *Immunosuppressive therapy during the first 20 days following transplantation (mean ± 1 S.D.)*

	ECIB	Local graft irradiation	Imuran® (mg/kg b.wt.)	Prednisone (mg/kg b.wt.)
Group I	67	2	1.9±0.5	2.0±1.8
Group II	7*	21	1.9±0.7	3.4±2.5
Difference		$p<0.01$	n.s.	

Only at rejection crises.

Table IV *Infections in groups I and II*

	Infection from: Ureteral leakage	Perigraftal hematoma	Focus unknown	Pulmonary infection	Severe mycotic infect.	Herpes zoster	Pulmonary tubercul.	Listeria meningitis
Group I	3	6	1	4	2	7		1
Group II	7	10	1	1	3	6		

maintain depletion, 3) as an aid in treatment of rejection crises.

In the present study ECIB has been given according to the first two recommendations to patients receiving necrokidney grafts. Despite a clear-cut reduction in the number of severe rejection epidoses only a 10% increase in graft survival was apparent in the treated group after 12 months and the result was not statistically significant.

There are several possible explanations for this apparent discrepancy between experimental and clinical results. The following two may be the most important.

Firstly, in the clinical studies ECIB was given in addition to conventional immunosuppression with azathioprine and prednisone, whereas these drugs were not given to the experimental animals. In clinical work one is thus studying the effect of ECIB superimposed on an immunosuppressive treatment of considerable efficiency.

Secondly, in clinical work with necrokidney transplantation there are obvious practical difficulties in timing pretreatment with ECIB in such a way that it is completely terminated shortly before grafting. In practice the interval between cessation of ECIB and grafting varied from 1 day to 18 months in the present material. Moreover, in several patients ECIB treatment was not completed when a graft became available. Taking these factors into account, the results illustrated in Fig. 5 indicate that a sufficent ECIB treatment increases graft survival by 25% after 1 year as compared to insufficiently ECIB-treated patients and to the untreated group. Although the apparent improvement is not statistically significant in the limited material studied, it cannot be excluded that the immunosuppressive effect of ECIB might be increased if ECIB is administered at more frequent intervals or alternatively if patients are transplanted as soon as possible after cessation of ECIB disregarding histocompatibility testing (which is apparently of limited value with current technique when necroorgans are used).

The effect of ECIB in production of lymphopenia in animals as well as in patients seems established (14). As described elsewhere the lymphocyte concentration cannot be reduced to more than 1/3–1/4 of pre ECIB level despite prolonged ECIB (14). Most of the remaining lymphocyte population is probably short-lived cells. It has been reported, however, that short-lived lymphocytes include B lymphocytes as well as T lymphocytes and that they are able to induce cell-mediated immunity (4, 5, 9). This finding is in accordance with the clinical results of the present study which clearly show that the quantitative and qualitative capacity of the reduced lymphocyte population in the ECIB-treated patients is sufficient for induction of the transplantation reaction. In view of this experimental and clinical evidence it cannot be excluded that the immunosuppressive effect of ECIB may be increased by additional treatment after grafting with antilymphocytic globulin, which damages the T lymphocytes more selectively (10).

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PARTIAL LIPODYSTROPHY AND CHRONIC HYPOCOMPLEMENTEMIC GLOMERULONEPHRITIS

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Abstract In partial lipodystrophy serious renal disease is common and often has the appearance of chronic hypocomplementemic glomerulonephritis. In this case report young woman with typical clinical history is described. Low serum levels for C3 were not affected by long-time immunosuppression nor was the presence of C3NeF The degree of renal insufficiency exerted no influence on C3. Transplantation resulted in normalization of C3 and disappearance of C3NeF persisting even in terminal graft failure.

The lipodystrophies are characterized by an atrophy of the subcutaneous adipose tissue and are, according to the distribution of this atrophy divided into partial and total forms Little is known about their cause nor is there full unanimity whether the various forms are distinct entities or more closely related The atrophy of the fatty tissue is in itself harmless but these disorders gain interest from the conspicuous coexistence of a number of more serious diseases In both the total and partial forms various cardiovascular cerebral hepatic, renal endocrine and metabolic disorders have been described reviewed by Senior and Gellis (17)

Renal disease in partial lipodystrophy occurs frequently in 25–50% of cases (1.., 20) and often proceeds rapidly to terminal renal failure Only a few extensively documented cases have been re ported and neither the pathogenesis of the renal disease nor the nature of ts connection with the fatty tissue disorder is understood.

We present a patient with partial lipodystrophy and chronic hypocomplementemic glomerulonephritis followed for two and a half years including periods of immunosuppressive treatment before and after transplantation.

METHODS

Serum hemolytic complement (total complemen) Sheep red cells were sensitized with amboceptor against the Forssmann antigen prepared in this laboratory the method described by Rapp (14). The total reac ol-ume in the immunohemolytic system was 1 3 ml on-sisting of 1.0 ml of 1% sheep red cell suspension 0 1 ml of the patient serum in serial dilution and 0.2 ml of an isotonic veronal buffer of pH 45 n-taining Ca^{++} and Mg^{++} ions. The reaction mix re was incubated at 37°C for 30 min. The degree of hemolysis in each test tube was calculated by measu g the content of Hb with Beckman B spectrophotom er at 541 mμ. The procedure for calculating the titre in 50% hemolysis units (CH_{50}) has been described previously (3)

Immunochemical titration of C3 The antiserum to C3 was prepared in rabbit by injecting C3 absorbed to zymosan according to the method described by Stratton (19). Analyses were performed by the Mancini technique (13). In order to facilitate comparison of titrations done on different dates, standards were always included on each plate.

The presence of the so-called C3N F (21) was determined by incubating equal amounts of the patient serum and normal serum at 37°C for 15 min followed by 45 min incubation at 37°C in the presence of 0 01 M Edetic acid (23). The conversion of C3 (B1C) to C3b (B1A) was then analysed by immunoelectrophoresis according to Scheidegger (16).

An antiserum to C4 was provided by Dr P Sjöström

CASE REPORT

A 28-year-old woman was admitted to our clinic in 1969 because of hypertension and raised serum creatinine Past history revealed that she had developed normally during her first years of lif (Fig. 1a) At the age of 3 she had severe pper respiratory tract infection and ver since slight proteinuria. During the years following and up to the age of 10 she developed progressing atrophy of the subcutaneous affecting the upper half of the body (Fig. 1b)



Fig. 1. The patient as a 1 year-old child (*a*), at the age of 10 after having developed partial lipodystrophy (*b*), 29 years old during treatment with corticosteroids (*c*).

trophy thereafter remained stationary. I.v. pyelography at the age of 19 was normal but serum creatinine was raised (1.7 mg/100 ml). Pregnancy and delivery two years later passed without complications and it was not until the age of 28 that she came under regular supervision. She was then found to have hypertension (210/170) because of marked proteinuria and a serum creatinine level of 2.6 mg/100 ml was transferred to our clinic.

On first admission her hypertension had already been treated and her BP was normal. She had a cadaverous appearance with lipodystrophy of the face, neck, arms and upper chest. There was no edema. Ophthalmoscopy disclosed hypertensive fundi grade II (Keith-Wagener). Blood Hb was 10 g/100 ml and ESR (Westergren) mm/1 h. There were normal WBC values, including differential count and platelets. The antistreptolysin-O titre was normal and the tests for antinuclear factors negative. Serum sodium was 138 mEq/l, potassium 4.7, calcium 4.5, chloride 106 and bicarbonate 4 mEq/l, serum phosphate 1.3 mM/100 ml, serum albumin 3.0 g/100 ml and total serum protein 6. g/100 ml. IgG was 530 mg/100 ml (normal range 756–1961), IgA 110 (normal range 82–185) and IgM 200 mg/100 ml (normal range 54–169). There was no clinical or laboratory evidence of thyroid or liver disease. The renal function had rapidly deteriorated over a few weeks. Serum creatinine had now reached 4.6 ml/100 ml. Serum urea was 85 mg/100 ml, inulin clearance 18 ml/min and PAH clearance 138 ml/min (filtration fraction 13%). Urinalysis showed numerous red and white cells but no casts. Urinary protein excretion was 5–10 g/24 h. Repeated urinary cultures were negative. Total hemolytic serum complement was 30 (normal range 45–135), C3 4.5 mg/100 ml (normal range 48–138) and C4 60% of normal value. C3NeF could be demonstrated.

The patient refused renal biopsy but the clinical picture resembled chronic hypocomplementemic glomerulonephritis and a trial with immunosuppressive treatment was started. She first received a daily dosage of 300 mg azathioprine and 4 mg of dexamethasone gradually tapered down to a maintenance dose of 100 mg and 1 mg respectively. At first there seemed to be some response since serum creatinine did not rise further for weeks (Fig.). However, serum complement levels were not affected and the proteinuria persisted. A progressive deterioration in renal function again started following upon an upper respiratory tract infection. The immunosuppressive treatment was continued for five months. Her lipodystrophy was not affected by this continued use of corticosteroids (Fig. 1c). Intermittent hemodialysis was begun 15 months after first

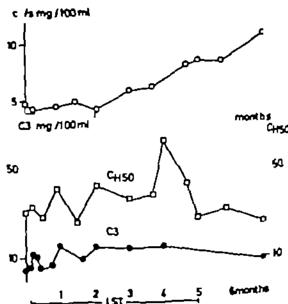


Fig. 2. The course after first admission showing persistent low serum levels of C_{H50} and C3 during immunosuppressive treatment (*IST*) and progressive uremia with a rising serum creatinine level (*cr/s*). C4 normal during the whole period.

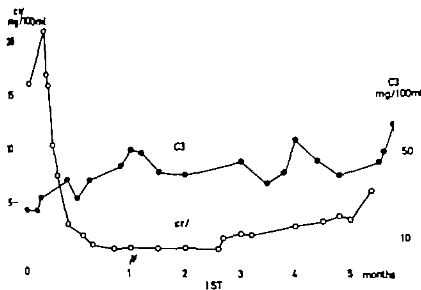


Fig. 3. Normalization of low C3 after kidney grafting. Abbr. as Fig.

admission. She became Australia-antigen-positive but there was no other clinical or laboratory evidence of hepatitis than a temporarily elevated S-GPT level, at the most 48 units (upper normal 30).

After 8 months of uneventful maintenance dialysis the patient received a cadaver kidney transplant with two HL A incompatibilities. Her own kidneys were not removed. Serum creatinine became normal within a month and C3 levels, which had been persistently low rose to normal values (Fig. 3). C3 thereafter remained normal or slightly subnormal during the whole course. C3NeF could no longer be demonstrated. Two months after transplantation she developed diabetes mellitus, requiring insulin and soon afterwards a picture of steroid-resistant graft rejection. Her renal function became successively impaired. The proteinuria amounted to about 1 g/24 h, no hematuria occurred. Five months after transplantation pericarditis supervened, she was returned to dialysis and transplantectomy was performed. She died on the second postoperative day. At autopsy her own kidneys were found to be small and shrunken with a non-specific appearance of end-stage kidney disease. Histological examination of the transplant revealed no sign of recurrent glomerulonephritis but only a picture of acute and chronic rejection. Other findings were an acute fibrinous pericarditis and severe atheromatosis, most pronounced in the coronary arteries. The subcutaneous tissue above the costal margin contained no fat cells, whereas below this the fatty tissue appeared normal.

DISCUSSION

Our case presented many similarities to others reported with renal disease in partial lipodystrophy. The disorder first appeared after a febrile upper respiratory tract infection. After a symptomless interval of several years signs of severe renal disease appeared. Heavy proteinuria and hypertension were part of the clinical picture and treatment with immunosuppressive drugs was of no apparent benefit.

The increased incidence of renal disorders in partial lipodystrophy was first described 15 years ago (4). These nephropathies often result in ... and pregnancies seem to be hazardous (2 ...), though not obviously so in our case. These renal disorders have long been believed to follow no particular clinical, histological or laboratory pattern (7). Most of the recently reported cases however indicate that there is a definite prevalence of glomerulonephritis. Membranoproliferative nephritis seems to be particularly common (8, 10, 1.., 15, 20, 4). Electron microscopy has revealed deposits within an unevenly thickened basement membrane (7). In the glomeruli immunoglobulins and complement have been found in a nodular pattern (8). In three cases (8, 4) in addition to our own complement analyses have been performed and all these patients had low C3 levels. It thus appears as if the renal disease in partial lipodystrophy often has the appearance of a hypocomplementemic glomerulonephritis with immune complexes deposited on the outside of the basement membrane. Like Williams et al. (24) we could demonstrate the presence of the so-called C3NeF (21), which disappeared in parallel with the normalization of C3 after transplantation.

Previous reports of partial lipodystrophy and renal disease have not dealt with serum

ment levels during prolonged immunosuppressive treatment and after renal transplantation. In the present case the hypocomplementemia persisted during five months of futile treatment with corticosteroids and azathioprine. The C3 levels were not influenced by the progress of renal failure. The transplantation of a cadaver kidney resulted in a rise of the serum C3 level, which remained normal also when ultimately the graft severely deteriorated in its function. Thus neither relief of uremia nor the posttransplantation drug treatment is likely to have caused the normalization of C3 in this patient.

Clinically the complement system has only been clearly shown to be of importance in a few disorders. It has, however, been discussed in connection with a number of so-called autoimmune disorders. The system may be activated in principally two ways. First along the classical pathway via antigen-antibody complexes as appears to be the case in SLE, and secondly, bypassing C1, 2, 4, through direct activation of C3 to C9 (6). There is evidence that the latter alternative bypass mechanism is the dominant pathway in acute streptococcal hypocomplementemic glomerulonephritis and in the so-called chronic membranoproliferative hypocomplementemic glomerulonephritis (5). Though the complement system can be manipulated in different ways in vitro, the significance of this in disorders involving the complement remains unclear.

The high frequency of renal disorders in connection with lipodystrophy is remarkable and an interesting hypothesis would be that the low C3 levels and lipodystrophy may both be due to a common metabolic defect, either genetic or acquired. For example it is possible to imagine that these patients readily develop a deficiency of some kind of inhibitor—as with KAF (konglutinogen activating factor) which, according to Lachman and Nicol (11) appears to inhibit C3b, which in turn perpetuates the breakdown of C3 in the alternative bypass system.

It is difficult to explain why C3 levels became normal in our patient after renal transplantation. In patients without lipodystrophy but who have chronic hypocomplementemic glomerulonephritis both normalization (22) and unchanged low (1, 9) complement levels have been reported. The association between lipodystrophy, chronic glomerulonephritis and hypocomplementemia would seem to indicate further detailed studies of the complement system in patients with lipodystrophy and renal disease as early as possible in the course of their nephritis, as well as in patients with no sign of renal disease. Such studies could possibly shed light on the pathogenesis of lipodystrophy as well as glomerulonephritis and improve our understanding of the way in which the complement system is involved.

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RENAL ARTERY OCCLUSION MALIGNANT HYPERTENSION AND THROMBOTIC THROMBOCYTOPENIC PURPURA

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Abstract. A case with unilateral renal artery stenosis and malignant hypertension, complicated with thrombotic thrombocytopenic purpura (TTP) is described The patient was uraemic due to severe arteriolar sclerosis in the contralateral kidney A massive consumption of platelets, decreased fibrinogen value and occurrence of fibrin/fibrinogen degradation products (FDP) in serum and urine were demonstrated. During treatment with heparin the renal function improved, the platelet count rose and the FDP amount in the serum as well as in the urine decreased. Reconstructive surgery of the occluded artery was then performed and the contralateral kidney was removed.

Thrombotic thrombocytopenic purpura (TTP) can occur as a complication to malignant hypertension and is probably caused by extensive microvascular lesions in this condition (15) The present case report concerns a patient with malignant hypertension due to unilateral renal artery stenosis with uraemia due to severe arteriolar sclerosis in the contralateral kidney and complicated with TTP The value of heparin treatment in such patients has been discussed (7 15) In the present case heparin treatment seemed to prevent the consumption of fibrinogen and platelets Since the renal parenchyma on the right (occluded) side was normal reconstructive surgery of this artery was performed. The contralateral kidney was destroyed by the prolonged and severe hypertension and was removed.

METHODS

The following laboratory studies and determination were made of the platelets, coagulation factors and components of the fibrinolytic system, platelet count, bleeding time (method of Duke), recalcification time of plasma, one-stage proth ombin tim ombin+ factor VII+factor X (Owren P&P c V factor VIII (biological activity and mical determination) fibrinogen spontane ac tivity of plasma and resuspended eu te on fibrin plates plasminogen (unitmoch I) fibrin/fibrinogen degradation product FL and rine inhibitors of plasminogen macroglobulin and ethanol gelation test I wise stated, the methods described earlier (6 10 12, 22 23)

Factor VIII was immunochemically determi cording to Holmberg and N lsson (14)

Ethanol gelation test was performed rdi Godal et al. (8)

Antithrombin III was immunochemically determined according to Hedner and Nilsson (11)

Typing of FDP in the urine was performed de scribed by Bouma et al. (4) and Hedner et al. (9)

Platelet survival studies wer performed as described by Ljungqvist and Bergentz (16). The Cr^{51} activity in platelet-rich plasma was measured repeatedly during 48 h after the infusion of Cr^{51}-labelled normal platelets.

CASE REPORT

A woman aged 48 had felt well until the end of 1971 when she progressively developed severe occipital headache and dizziness. Two month before admission she developed shortnes of breath and swelling of the legs. She was admitted to the hospital with nausea, vomiting and blurred vision

On admission the patient was tired and pale but alert and cooperative She had peripheral oedema and icteric sclerae Petechiae wer seen on the legs. The liver could be palpated but there was no splenomegaly BP was 250/110 mmHg. Routine neurological examion revealed nothing remarkable Papilloedema, rhages and xudates in her fundi were demon

Laboratory finding Hb 9 7 g/100 ml, ferential count normal platelet count



Fig. 1. Renal arteriogram showing occlusion of right renal artery with collateral vessels to the small kidney. The left renal artery is normal but there is no visualization of the small intrarenal branches on this side.

raised, serum electrophoresis showed inflammatory
ction, but the γ-globulins were essentially normal.
oglobin 7 mg/100 ml. The blood film revealed
ytosis and poikilocytosis and fragmented red
s. Bone marrow contained increased megacaryocytic
d erythrocytic series. The direct and indirect Coombs tests were negative. LE cell preparations were negative. Bilirubin 1.6 mg/100 ml with positive direct reaction. Serum creatinine 2.2 mg/100 ml. Urinalysis showed proteinuria of glomerular unselective type and haematuria.

X-ray examination. Enlargement of the heart and pulmonary congestion were found. Aortography and selective renal arteriography showed total occlusion of the right main renal artery with multiple collateral vessels to an abnormally small kidney. The left kidney was slightly larger than normal. The main renal artery to that kidney was intact but the small vessels of the cortex were markedly narrowed (Fig. 1).

Coagulation studies on admission (Table I). The patient had marked thrombocytopenia, but Duke's bleeding time was normal. The AHF level determined immunochemically was as high as 600%; the biological AHF activity was about one third as high, namely 250%. Factor V was increased but P&P and fibrinogen were normal. The ethanol gelation test was negative. The various components of the fibrinolytic system in the patient's blood were essentially normal, but high

Table I. *Coagulation and fibrinolytic factors on admission*

		Normal range
Bleeding time (min)	5	1–5
Platelets/mm³	70 000	150 000–400 000
Recalcification time (sec)	112	112–195
One-stage prothrombin time (sec)	12	14–16
P&P (%)	100	80–120
Factor V (%)	142	80–120
Factor VIII (biol.) (%)	250	60–160
Factor VIII (immunochem.) (%)	600	60–160
Fibrinogen (g/100 ml)	0.47	0.20–0.40
Antithrombin III	128	60–140
Spontaneous fibrinolytic activity (lysed area in mm²)		
Plasma	0	0–50
Resusp. euglob. prec.	0	0–70
Plasminogen (%)	170	60–140
FDP in serum (μg/ml)	40	0–5
FDP in urine (μg/ml)	27	0
Inhibitors of plasminogen activation (urokinase inhibitors) (%)	174	60–140
α_2-macroglobulin (%)	140	80–120
Ethanol gelation test	Neg.	Neg.

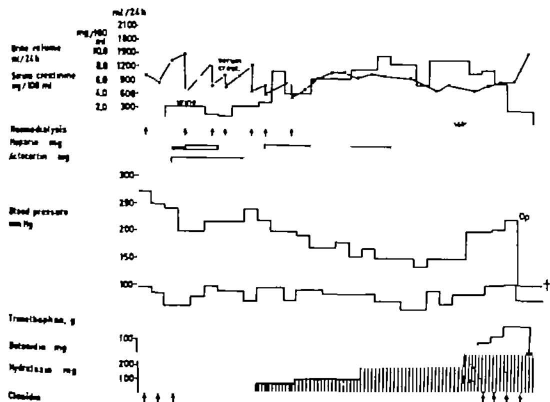


Fig 2 Treatment, urinary output, serum creatinine and BP throughout the disease.

molecular weight FDP in the serum and urine were increased. Platelet survival with Cr^{51}-labelled normal platelets showed a half-lf of about 30 min .g. marked shortening.

Further course After 2 3 days the patient condition deteriorated, with fluctuating neurological symptoms, and she became intermittently comatose. Paresis of the left arm and leg and left facial palsy developed Babinski' sign was elicited bilaterally

Haemodialysis was begun because of oliguria with grave electrolyte deficits and pulmonary oedema. BP did not improve during the dialysis and control of the pressure required continuous infusion of trimethaphan (Arfonad®). A continuous heparin infusion was started and the platelet count rose to 110000 after 2 days (Fig 3) During the following 3 days heparin was withdrawn, the platelet count decreasing to 15000 as result The platelet count returned to normal during heparin infusion, which was continued for 5 weeks. During this period the fibrinogen level rose, FDP in serum and urine decreased and AHF and factor V decreased toward normal values (Fig. 3) Hydrocortisone (Actocortin®) 100 mg×4 was given from the beginning of treatment. The patient improved during dialysis, but the hemiparesis of the left side persisted. After 6 haemodialyses treatments the urinary output increased and renal function improved, but the serum creatinine persisted at level of 5–6 mg/100 ml. BP was under control during treatment with bethanidine (Esbatal®) and hydralazine (Apresolin®).

Further studies of renal function were now made. The uraemia indicated that the parenchyma of the left kidney must be seriously damaged. This was confirmed by xamination of biopsy specimen of that kidney which showed severe arteriolar sclerosis. After temporary improvement the patient condition deteriorated and signs of pneumonia developed BP rose in spite of excessive antihypertensive treatment and the patient had episodes of cardiac arrest. In an attempt to control the BP and the uraemia, the right renal artery was repaired and the contralateral kidney was removed. A peroperative biopsy specimen of the right kidney showed essentially normal glomeruli and vessels. BP became normal after the operation and the urinary output from the reconstructed kidney was satisfactory but the patient died from new cardiac arrest during insertion of pacemaker

Pathological examination

The biopsy specimen of the left kidney showed severe arteriolar disease with profuse ifération

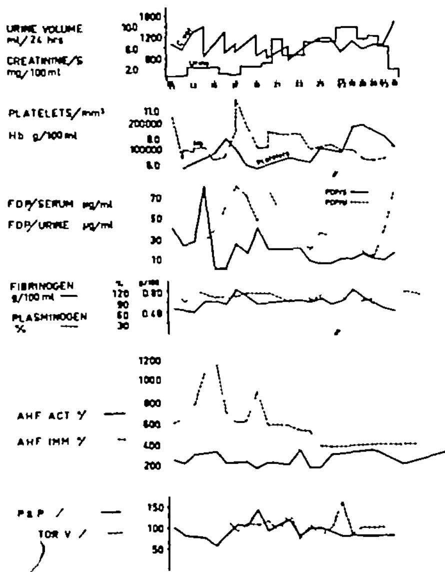


Fig. 3. Pattern of the coagulation and fibrinolytic components during the disease

fibrinoid necroses. The lumina of these vessels were very narrow and in occasional areas completely obliterated. Some of the glomeruli had undergone hyalinization, others were necrotized and the basement membrane of the glomerular capillaries was thickened. The main left renal artery was intact.

Examination of the right kidney showed that the glomeruli and tubular epithelium were well preserved. Hyperplasia of the juxtaglomerular apparatus was present. No arteriolar changes were seen. The main renal artery was occluded by a thrombus which partly organized. Changes in the collagen of the media suggested fibrous dysplasia.

DISCUSSION

On admission the patient had malignant hypertension with involvement of various organs. The hypertension was due to an occlusion of the right renal artery as confirmed by the hyperplasia of the juxtaglomerular cells. The kidney volume was reduced and a number of collaterals had developed. The patient was known to have had symptoms of hypertension for at least one year. The aorta was not arteriosclerotic and the left renal artery was intact. This argues against arteriosclerosis as a cause of the stenosis. Fibromuscular hyperplasia, progressing to a total occlusion, is a more probable explanation. This disease is most common in middle-aged women. The aetiology of the condition is not known ( ). The pathology of this disease varies. In our case the media of the vessel wall was involved.

It is known that severe hypertension due to stenosis or occlusion of a renal artery may dis-

appear if the arterial lesion is repaired (20). In severe and prolonged hypertension the contralateral kidney may undergo changes resulting in persistence of the hypertension after arterial reconstruction, while the kidney on the stenotic side is protected (17 20 27). Removal of the 'unprotected' kidney might then have a favourable effect on the hypertension (27). Biopsy specimens of the kidneys of our patient showed severe damage in the unprotected kidney but nothing remarkable in the constricted kidney on the stenotic side. No determination was made of the split renal function or separate renin activity in this patient because of her poor general condition.

In this case the malignant hypertension was complicated by thrombocytopenia haemolytic anaemia, fluctuating neurological signs and uraemia findings typical of TTP (21). The peripheral blood smear and the findings in the bone marrow confirmed the diagnosis. Malignant hypertension has been suggested to initiate abnormalities in the microcirculation, and thereby damage on the vessel walls with the development of a disseminated intravascular coagulation and microangiopathic haemolytic anaemia as a result (15). In our patient the Duke bleeding time was normal in spite of the low platelet count. Remarkably enough, the fibrinogen was normal in this highly reactive condition. This may indicate a rapid consumption of fibrinogen by coagulation and/or fibrinolysis. Also platelet survival studies showed that the turnover was very rapid the half-life being only 1/2 hour indicating a massive consumption of platelets. The AHF and the AHF-related protein obtained immunologically were markedly increased as was the factor V a it often is on activation of the coagulation process. The patient had high concentrations of high molecular weight FDP in urine indicating renal damage with fibrin deposits (5 9). The results of these analyses agree with those reported in TTP (1 18 19).

The therapy used in this disorder includes heparin (3 24) large doses of steroids (13) splenectomy (13) haemodialysis (26) and transfusions of fresh blood (25). In our case determinations of platelets coagulation factors and FDP indicated that the heparin given prevented the consumption of the coagulation factors.

It seems that the patient had had hypertension for a long period, which resulted in destruction of the small vessels and microthrombosis causing the TTP which probably aggravated the hypertension and organ damage. As mentioned above treatment with heparin combined with haemodialysis appears to be indicated in this often fulminating and fatal disease. As expected no signs of microthrombosis were found post mortem because the thrombi must have been dissolved by the time of death almost months after the symptoms of TTP had disappeared.

ACKNOWLEDGEMENTS

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CARDIOVASCULAR EFFECTS OF POISONING WITH TRICYCLIC ANTIDEPRESSANTS

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Abstract. Cardiovascular dynamics have been studied in 10 patients admitted because of poisoning by tricyclic antidepressants (TCA) and in 7 with hypnotic drug (HD) poisoning. Right heart catheterization (floating catheter technique) and determination of cardiac output (dye dilution technique) have been performed during and after an initial comatous phase. In the TCA group there were marked ECG changes with a mean increase of QRS duration to 0.14 sec during the comatous phase. The cardiac output was then increased in relation to the calculated oxygen uptake compared with the findings in awake state when the ECG changes had diminished or disappeared. After i.v. administration of 5 mg propranolol the fall in cardiac output was most pronounced during the comatous phase but the broadening of QRS complexes was not affected. The central venous and systemic arterial BP were normal. As judged by the normal diastolic pressure in the pulmonary artery and the normal central venous pressure there were no signs of myocardial insufficiency. The cardiac output in the TCA group was twice as high, i.e. significantly higher, as in the HD patients. The arteriovenous oxygen difference was significantly lower (on an average 40%). The hyperkinetic circulation during TCA poisoning may be explained by a dominant adrenergic stimulatory effect on the circulation.

Tricyclic antidepressants (TCA) are of great value in the treatment of depressive states and consequently the use of these drugs has increased steadily since their introduction about 20 years ago. As might be expected the number of poisonings has also increased and the outcome after excessive doses of TCA may be fatal as a rule because of cardiovascular complications. Some adverse side-effects, particularly from the cardiovascular system have been reported also within the therapeutic range (22). Amitriptyline and imipramine are reported to have a high affinity for the myocardium (11, 16).

Most reports have been focused upon the ECG changes (3, 18) comprising arrhythmias, widening of the QRS complex and marked changes in the ST and T segments. In this respect TCA differ from other drugs with psychotherapeutic sedative effects. ECG changes are constantly found in cases of moderate to severe poisoning with TCA. Less pronounced ECG changes have been reported after poisoning with the chemically closely related phenothiazines, especially thioridazine (2). Depressive effects on the myocardium have been found in animal experiments (4). However there are no reports of hemodynamic studies in man during poisoning with TCA. The action of TCA on the heart and circulation is still obscure.

The purpose of the present study was to report and analyse some circulatory data in patients with pathological ECG changes following ingestion of large doses of TCA for suicidal purpose. Such data are urgently needed in order to obtain a rationale for the therapy of TCA poisoning.

MATERIAL

TCA patients. Ten patients, severely poisoned by ingestion of amitriptyline and in one case nortriptyline were included in the study. Their ages varied from 25 to 50 years (mean 32). Further clinical data are presented in Table I. In six of the patients other drugs than TCA had also been ingested, as revealed by extensive toxicological analyses of blood and urine. Concentrations of drugs other than TCA were in general low. All patients had ECG changes characteristic of TCA poisoning, which obviously dominated the clinical picture. On admission all patients were comatose. The mean duration of coma was 35 hours. In two cases the approximate time of poisoning was unknown and

Table I Clinical data of the patients

Case no.	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m^2)	Drugs ingested	Dose (mg)	Duration of coma (h)	Max. QRS time (sec)
TCA patients (=10)									
1	♂	29	160	40	1.51	Amitriptyline (Triptyl forte)+ chlorprothixen (Truxal®)	≤5 000	60	0,19
2	♀	30	162	45	1 45	Amitriptyline (Tryptizol®)	2 500	60	0 14
3	♂	?9	185	96	2.20	Amitriptyline (Tryptizol®)	2 500?	30	0,12
4	♀	??	167	57	1 64	Amitriptyline (Tryptizol®)	≤2 500	15	0 14
5	♂	?5	175	70	1.86	Nortriptyline (Aventyl®)+ diazepam (Valium)	2 500?	25	0,1?
6	♀	50	155	46	1.53	Amitriptyline (Laroxyl)+ protriptyline (Concordin®)+ methaqualon (Mandra ®)	≤2 500 ≤1 000	20	0 13
		41	167	60	1 67	Amitriptyline (Tryptizol®)+ barbiturate	?	25	0,13
		35	183	83	2.06	Amitriptyline (Tryptizol®)	600	15	0 13
		?7	162	5?	1.55	Amitriptyline (Tryptizol®)+ fluphenazine (Pacinol®)+ diazepam (Stesolid®)	2 000	40	0 16
10			167	66	1 74	Amitriptyline (Tryptizol)+ levopromazine (Nozinan®)	?	60	0 22
HD ( )									
11				5?	1.58	Butenemal (Dimunal®)+ nitrazepam (Mogadon)	1 200 150	36	0.09
12				6?	1 73	Glutethimide (Doriden)	19 000	34	0 08
13				70	1 87	Chlorpromazine (Hibernal®)	?	72	0 11
14				75	1 86	Butenemal-allypropymal (Dimimal duplex)+alcohol	?	72	0 13
15				?9	1 47	Propiomazine (Propavan)	≥2 500	24	0 08
16				?0	1 90	Butenemal-allypropymal (Diminal duplex)	15 000	90	0,08
17	♂				1 73	Meballymalnatr -allyl-bromallylbarb -hydroxyzine (Vesparax)	?	55	0,08

4

mated from bne l data S patient received ment of positive pressure entilation with Engström respirators The initial investigation performed on an average 21 hours after drug ingestion hile the patients were till comatous The second investigation was performed on an erage 37 hours later when the patients were awake They had not ecovered completely however and some ECG changes remained The average QRS duration was at the upper normal limit The hemodynamic effects of TCA poisoning ha e been compared with the effect of mainly hypnotic drug (HD) poisoning in corresponding clinical phases

HD patients This material consisted of 7 patients 4 men and 3 women, of whom 5 have been included in an earlier report (6) (Table I) Their mean age was 44 years The total duration of coma as 52 hours The time between drug intake and the initial hemodynamic investigation was 20 hours and the time between the initial and final determination 45 hours.

The body temperature during the initial investigation veraged 35.3°C in the TCA and 33.6°C in the HD material The difference in temperature was not statistically significant. The temperature during the fir investigation was 37 3 and 37 7°C respectively

METHODS

The methods used have earlier been reported in deta (6). Right heart catheterization was performed by th floating catheter technique. Cardiac output was dete mined by the dye dilution method using cardiogree as indicator The present investigation also include determination of the maximum first derivative of th systemic arterial pressure, $(dP/dt)_{max}$ derived fro radial artery pressure curves in four patients (7). Th catheter was connected to Hewlett Packard 12 transducer which in turn wa connected to Sanbor 311 amplifier The output of this amplifier was co nected to the input of a Philips EL 1020/07 tape re corder The catheters were always of the same leng and diameter and there was an approximately flat frequency response up to 20 Hz. The data wer analysed "off line" on an IBM 1800 or 360/75 com

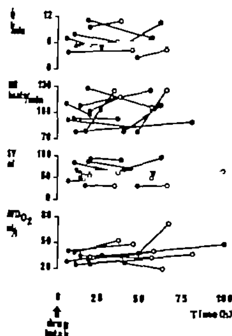


Fig. 1 Cardiac output ($\dot{Q}$), heart rate (*HR*), stroke volume (*SV*) and arteriovenous xygen difference ($AVDo_2$) in relation to time courses in 10 TCA patients. ●=comatose, O= wake.

puter (5). Four patients (nos 5 8 9 10) investigated befor and about 10 m after sec tion of 5 mg propranolol both in comatou and wake states. Repeated diagnostic ECG recordings ere taken in all patients. The xcretion of catecholamines in th urine was determined fou patient Besides sinus tachycardia and transitional entricular ectopic beat induced by the cardiac passage of he catheter no arrhythmias or other complications occurred during the investigations

RESULTS

The hemodynamic data obtained i h ten TCA patients are shown in Table II Fou the hemodynamic variables are presented in F 1

ECG Prolongation of the QRS t me he most characteristic ECG change in th es wa present in all cases (Fig 2) The max mal e corded QRS time was on an erage 0 16 ec (range 0 12–0.22) and at the time of h f t hemodynamic investigation 0 14 sec (ran 0 18) At the final investigation when the at t were awake the QRS time was 0 11 sec n e cases (nos. 1 8 and 10) but did not exceed upper normal limit of 0 10 sec in the remainder

Heart rate was elevated above normal lim for resting individuals It was 97 beats/min (range

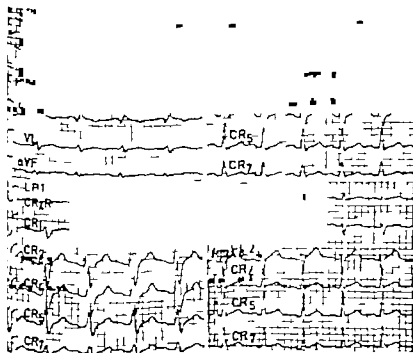


Fig 2 Typical ECG changes in case 10. One week had elapsed between the initial (left) and the final (bottom right) recordings.

Table II *Data obtained in connection with right heart catheterization during and after coma in 10 TC[A] patients and 7 HD patients*

c=coma, =awake HR=heart rate Q̇=cardiac output AVD_{O_2}=arteriovenous oxygen difference, $\dot{V}_{O_2}$=oxygen up take, SV=stroke volume CV=central enous, RA=radial artery PA=pulmonary artery S=systolic, D=diastolic M=mean R_s=systemic vascular resistance

Case no.	Body temp (°C)	HR (beats/min)	Q̇ (l/min)	AVD_{O_2} (ml/l)	$\dot{V}_{O_2}$ (ml/min STPD)	SV (ml)	Pressures (mmHg) CV	RA S	RA D	RA M	PA S	PA D	PA M
TCA patient													
1 c	33 1	79	5 6	28	155	71	3	129	81	100	22	15	17
a	36 0	108	10.5	21	221	97	3	117	66	83	31	16	4
2 c	29 2	79	2.6	39	101	33	2	102	59	79	15	5	10
a	36.8	125	4 1	73	300	33	2	113	73	85	19	6	11
3 c	37 0	102	9 7	26	250	95	3	154	89	103	26	12	18
	38 0	118	10 9	31	334	92	3	154	97	112	23	12	16
4 c	36.6	110	7 0	28	195	68	3	130	75	97	20	10	14
	37.3	98	5.5	34	186	56	3	134	68	85	17	6	10
5 c	38.5	128	11.[illegible]	32	363	88	3	111	59	75	25	14	19
a	37.9	106	7 1	41	289	67	0	102	62	74	20	7	14
6 c	35.2	91	3.8	41	155	4	4	110	68	86	19	8	13
	37.3	84	4.3	50	216	51	3	122	65	84			
7 c	33.6	78	5.3	25	13[illegible]	67	2	119	66	92	20	7	12
	36 7	90	6 7	38	254	74	4	129	69	93	25	10	16
8 c	36.5	93	7 9	39	307	85	1	104	59	77	18	6	11
a	37 7	93	6 1	54	329	66	1	130	80	97	13	5	9
9 c	36.8	108	5.5	36	199	50	3	131	83	100	18	8	12
a	38 2	125	6 4	34	219	51	4	139	72	93	77	12	17
10 c	36.9	104	5 0	35	176	48	6	135	90	107	33	19	[illegible]
	–	103	5 6	49	272	54	2	138	89	107	32	17	22
patients													
c	34 0	90	2.7			54	6	115	90	99			
a	36.8	79	4 3	37	157	55	7	95	42	56	12	4	8
c	34.2	70	3 0	35	106	43	6	132	70	95	24	11	15
a	38.[illegible]	98	6.3	31	197	64	0	125	67	88	16	3	7
13 c	31 0	79	4 0	38	152	51	2	125	74	93	14	8	10
a	37.2	86	8.5	50	421	99	4	138	76	100	23	9	16
14 c	32.8	59	4 4	38	167	75	3	90	39	56	19	7	13
a	37 0	86	7 9	32	255	91	3	131	51	78	22	6	12
15 c	33 9	79	2.5	61	152	32	−1	97	60	75	15	7	9
a	37 4	113	5 3			47	7	113	62	78	20	9	13
16 c	33.5	72	2.6	61	159	36	4	95	65	76			
	38 9	142	10.8	33	358	76	3	108	59	73	25	12	17
17 c	35 9	68	2.9	56	164	43	5	101	60	74	20	9	14
a	38.5	98	5.3	45	241	54	2	135	71	93	25	6	13

78–128) during coma and in the awake state 105 beats/min (range 85–125) The difference was not significant.

Cardiac output averaged 6.4 l/min (range 2.6–11.2) during coma and 6 7 l/min (range 4 1–10 9) in the awake state. The difference was not significant

Stroke volume was essentially unchanged It was 65 ml (range 33–95) during coma and 64 ml (range 33–97) in the awake state

Arteriovenous oxygen difference was normal or low during coma and increased from 33 (range 25–41) to 43 ml/l (range 21–73) after coma. The difference was significant on the 5% level.

[illegible]	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	BE (mEq/l)	pH	Respirator treatm.
[illegible]	140	26	−2	7.48	+
[illegible]	190	54	−2	7.29	−
[illegible]	119	27	−2	7.51	+
[illegible]	71	37	+2	7 43	−
[illegible]	94	35	−5	7.35	−
[illegible]	90	37	−2	7.39	−
[illegible]	83	44	−1	7.36	−
[illegible]	82	36	+2	7 46	−
[illegible]	60	29	−11	7.33	−
[illegible]	75	37	−1	7 40	−
[illegible]	100	29	−1	7 47	+
[illegible]	69	38	+3	7 46	−
[illegible]	90	41	+	7 43	−
[illegible]	70	37	+2	7 45	−
[illegible]	61	33	−4	7 40	−
[illegible]	59	38	−1	7 40	−
[illegible]	90	34	0	7 45	+
[illegible]	63	31	−3	7 43	−
[illegible]	102	36	0	7 42	+
[illegible]	77	33	0	7 45	
[illegible]	169	31	−4	7 41	−
[illegible]	105	35	−3	.40	−
[illegible]	90	35	−7	7.33	+
[illegible]	68	36	−1	7 41	−
[illegible]	91	3[illegible]	−4	7 40	−
[illegible]	97	34	+2	7 48	−
[illegible]	105	43	+3	7 42	+
[illegible]	52	33	0	7 45	−
[illegible]	69	47	+4	7 40	−
[illegible]	72	39	0	7 41	−
[illegible]	47	30	+1	7.50	+
[illegible]	57	34	−1	7 42	−
[illegible]	78	34	+7	7.54	+
[illegible]	72	39	+1	7 43	−

Oxygen uptake was calculated from cardiac output and arteriovenous xygen difference and showed a probably significant increase from the comatous to the awake state ($p < 0.05$).

Intracardiac and intravascula press re During coma the pressures in the radial artery were normal except in one case (no 3) i whom it was

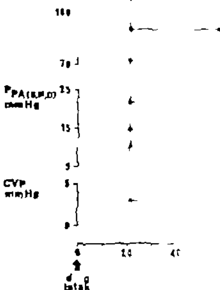


Fig 3 Systolic and di ±S E.M) in the adial artery P (P) and central e (CVP) el es times of in estigation in 10 TCA pati in Fig 1

slightly elevated After coma the diastolic and mean radial artery pressures were essentially unchanged. The systolic pressure increased by 14% which was not significant (Fig. 3) The pressure in the pulmonary artery was normal both during and after coma, except in patient 10 in whom it was slightly elevated. The central enou pressure was normal and remained essentially unchanged.

The maximum first derivati increased in all of the four cases nvestigated from an average of 1 02 to 1 76 mmHg msec after the coma (Fig. 4) The calculated peripheral vascular resistance was mainly unaffected as there was only minimal change of the cardiac output and mean arterial pressure

Arterial blood gases pH was unchanged between the two investigations and there were no significant changes in PaO_2 $PaCO_2$ and base excess

Effect of β-adrenergic eceptor blocking 1 four cases 5 mg propranolol (Inderal®) wa injected i.v The results are illustrated in Tabl and Fig 4

Following njection there was a decr

Table III *Hemodynamic effects of propranolol during and after coma in 4 TCA patients*
B=before A=after 5 mg propranolol, other abbreviations as in Table II

Case no.		Body temp (°C)	HR (beats/min)	Q (l/min)	AVD_{O_2} (ml/l)	V_{O_2} (ml/min STPD)	SV (ml)	Pressures (mmHg) CV	RA S	RA D	RA M	PA S	PA D
5	c B	38.5	128	11.[illegible]	3[illegible]	363	88	3	111	59	75	25	14
	c A		94	5 7	52	296	61	1	113	73	84	28	18
	B	37 9	106	7 1	41	289	67	0	102	62	74	20	7
	a A		94	6.8	45	303	72	4	103	59	73	22	10
8	c B	36.5	93	7 9	39	307	85	1	104	59	77	18	6
	c A		85	6.3	47	293	74	4	105	64	79	22	10
	B	37 7	93	6 1	54	329	66	1	130	80	97	13	5
	a A		84	5 1	58	[illegible]93	61	2	1[illegible]4	76	9[illegible]	16	5
9	c B	36.8	108	5.5	36	199	50	3	131	83	100	18	8
	c A		86	3.8	55	[illegible]07	45	5	113	75	89	19	9
	a B	38 2	125	6.4	34	[illegible]19	51	4	139	72	93	[illegible]7	12
	a A		106	4.5	49	[illegible]21	4[illegible]	6	121	68	85	26	13
10	c B	36 9	104	5 0	35	176	48	6	135	90	107	33	19
	c A		88	3 4	48	164	39	8	113	80	91	28	18
	a B		103	5 6	49	252	54	2	138	89	107	3[illegible]	17
	a A		91	3 9	59	230	43	5	122	84	101	35	21

heart rate and cardiac output and an increase of the arteriovenous oxygen difference in all cases both during and after coma The decrease of the art rate during coma was 20 and after coma eats/min. The decrease of cardiac output was and 1 l/min pectively The corresponding anges of strok olume were 13 and 5 ml The systemic arteri BP was essentially unaffected Thus the peripheral vascular resistance increased This increase was more pronounced during than after coma. The changes were 4.3 and 2 8 U respectively During and after coma the increase of the central venous pressure was respectively 4 0 and 2.5 mmHg and that of the mean pulmonary artery BP 1 5 and 2.8 mmHg. The diastolic pulmonary artery BP increased 2 mmHg at both investigations

Catecholamines in the urine In two of four cases (nos. 4 and 10) the maximal urinary excretion of norepinephrine was 86 and 98 μg/day respectively which is about double the normal value. In two cases (nos 5 and 8) the excretion was normal

Hypnotic drug poisoning The results obtained in seven cases of HD poisoning are presented in Table II A systematic comparison with the cor responding values for TCA patients (Table II reveals the following significant differences. I HD patients the heart rate and cardiac outpu were significantly lower ($p<0$ 001 and $p<0.0$ respectively) during coma than in TCA patient (Fig. 6) In patients with HD poisoning there wa a significant increase in heart rate and cardia output ($p<0$ 01) from the comatous to th awake state The arteriovenous oxygen differenc was significantly higher during coma in patient with HD than with TCA poisoning ($p<0$ 01) There was no significant difference in stroke vol ume between the two groups Peripheral vascula resistance was significantly higher ($p<0.02$) i the HD than in the TCA group during coma an decreased significantly ($p<0$ 01) from the coma tous to the awake state The calculated oxyge uptake increased from the comatous to the awak state both in the HD ($p<0$ 02) and the TC group ($p<0$ 05) and there was no significan difference between the two materials. The QRS time of 0 13 sec in case 14 (Table I) in the HI material was recorded immediately after a shor initial heart standstill Less than a day later the

I, ...	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	BE (mEq/l)	pH	Respirator treatm.
41	60	39	−11	7.33	−
76	54	29	−8	7.36	
84	75	37	−1	7.40	−
81	83	38	+1	7.43	
51	61	33	−4	7.40	−
85	67	34	−5	7.37	
87	59	38	−1	7.40	−
76	62	39	−1	7.39	
76	90	34	0	7.45	+
—	78	32	0	7.46	
81	63	31	−3	7.43	−
76	61	32	−3	7.4[illegible]	
3	107	36	0	7.42	+
74	147	33	−3	7.41	
16	77	35	0	7.45	
46	71	36	+1	7.44	

QRS time was 0.08 sec. Also with the higher the included the mean QRS time was normal (0.09 sec) compared with 0.14 sec in the TCA material. The slightly prolonged QRS time in case 6 is probably an effect of the phenothiazine drug. In the awake state there were no significant differences of hemodynamic variables between the two groups.

DISCUSSION

The finding of a lowered arteriovenous oxygen difference and a high cardiac output in this study of TCA poisoning has not been reported earlier. The results also show that there is a hyperkinetic circulation during the comatous phase of TCA poisoning compared with patients suffering from [illegible] overdose.

This difference cannot be explained by the difference in body temperature or other differences in the two materials except the kind of drug ingested. In the present material amitriptyline was the dominant drug. However clinical findings as well as the principal mode of action speak in favour of similar clinical and hemodynamic effects of other derivates within the TCA group (10, 20, 23). There was no difference in the ECG or hemodynamic pattern between the pure TCA poisonings and those combined with other drugs. Sigg et al (24) reported that cardiac output decreased after injection of 5 mg imipramine/kg [illegible] action of TCA has been dem[illegible] investigators (9, 1[illegible] 4). Thu[illegible] are adrenergic effects while [illegible] these effects are blocked pos[illegible] the adrenergic recept[illegible] are characterized by [illegible] arterial hypotension. Th[illegible] cardiotoxic effect and th[illegible] arrhythmias may al[illegible] this material may be [illegible] severely poisoned [illegible] adrenergic depression.

TCA are easily bound [illegible] and the body tissues. The ser[illegible] always be low. Borgå et al (8) d[illegible] isotope technique that the percentage [illegible] amitriptyline was 3.6 at therapeutic drug concentration of 0.29 µg/ml. They also found that the unbound fraction of desmethylimipramine in

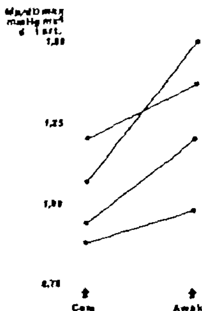


Fig. 4. Changes of the maximum [illegible] the radial artery pressure during and TCA pat ent

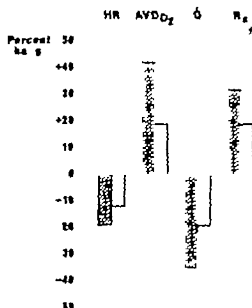


Fig 5 Relative changes of heart rate arteriovenous oxygen difference cardiac output and peripheral vascular resistance (R_s) 10 min after i. injection of 5 mg propranolol during (▨) and after coma (☐) in 4 TCA patients. Abbreviations as in Fig. 1

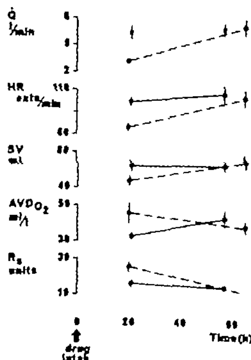


Fig 6 Mean values of cardiac output heart rate, stroke volume arteriovenous oxygen difference and peripheral vascular resistance in 10 TCA patients (—) and 7 HD patients ( ) in relation to the mean times of investigation. Abbreviations and symbols as in Fig. 1

plasma increased only twofold in vitro when total concentration of the drug was increase thousand times This implies only a minor incr of unbound TCA in poisonings with toxic pl levels In vivo the therapeutic plasma leve TCA seldom exceeds 0.3 μg/ml (13). Von E (1) found a high liver clearance of nortripty when orally administered to rats He concl that the drug seems to be concentrated and ra ly metabolized by the liver

According to Cairncross (9) and Theobald et (25) the autonomic effect of amitriptyline is c acterized by a strong anticholinergic action a relatively weak norepinephrine-potentiating eff Bevegård (4) demonstrated in man that the a cholinergic drug methylscopolaminenitrate had effect on cardiac output although heart rate creased markedly Concerning the cardiac ef in this material the direct adrenergic effect p dominates as reflected by the hyperkinetic circ tion Both central and peripheral effects have b described in TCA poisonings (14)

The fall in BP was not significant in the pres material but hypotension is a frequently obser cardiovascular effect (23 4 26) Generally att tion has been focused upon the depressive act on the heart However a peripheral vasodilatat effect of the drug with subsequent increase cardiac output could explain the hyperkinetic culation in this material Alternatively it might compatible with the findings that a supply imipramine increases the blood levels of ca cholamines (15) and that the catecholamine re take is inhibited after administration of TCA, amitriptyline (10) The few determinations of c cholamines in the urine in this material do offer any certain conclusions as to the possibi of increased catecholamines in the blood stream

Following i v injection of propranolol cardiodepressive action was most pronounced d ing coma. This further supports the view t TCA has a dominating adrenergic effect T occurs in spite of marked ECG changes with p longation of the QRS time The stress of a hyp kinetic circulation can thus be counteracted administration of β-receptor blocking agen Beneficial effects of β-receptor blocking age on TCA-induced cardiac arrhythmias have a been reported (17 19). However the danger myocardial insufficiency must be borne in and it is possible that a certain adrenergic

is appropriate to counteract a supposed direct side effect on the heart.

Evaluated from the diastolic pressure in the pulmonary artery there was no left ventricular insufficiency. Also the filling pressure of the right ventricle was normal. Only bedside X-rays of the chest were performed, which do not permit adequate determination of heart size.

The stroke volumes were in general somewhat small. The significance of this is not clear but a toxic effect on the myocardium as well as an anticholinergic effect cannot be excluded. The peripheral vascular resistance was unchanged between the two investigations. Therefore changes in the cardiac function in the same individual should be reflected in a peripheral pulse wave characteristic like $(dP/dt)_{max}$. The value was lowest during the coma, when pathologically broad QRS complexes were present. A slowed mechanical heart contraction reflected in decreased $(dP/dt)_{max}$ may be due to electromechanical dysfunction with prolonged depolarisation. This finding is possibly an expression of a supposed cardiotoxic effect of the drug. In spite of that the cardiac output was not impaired which is obviously explained by an increased adrenergic activity.

Nymark and Rasmussen (21) reported that pretreatment with propranolol prevented the occurrence of TCA-induced ECG changes in rabbits. They concluded that the most likely mechanism l the production of the ECG changes seems to be a heavily disturbed autonomic balance with an adrenergic dominance. In cases already poisoned the QRS complexes remained broad, which is in accordance with the findings in the present material. The mechanism of the alterations of intracardiac conductivity is still disputed (20). Metabolic disturbances possibly involving the cellular exchange of electrolytes ha e also been proposed.

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ACUTE INTOXICATION

A Comparative Investigation at a General City Hospital for the Years 1951 1961 and 1971

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ktract. Investigation of cases of acute int xication, dmitted to the Department of Internal Medicine, Helsgborg Central Hospital, in 1951 1961 and 1971 re aled that the number of cases had increased from 42 1951 to 230 in 1971 and that the frequency of cases r total number of inpatients at the Department had inased from 2.2% t 7.6% during the same time. Intocation with more than one agent had become more ommon, as had the combination medical drug+alcohl. The duration of hospital care was roughly the me in 1951 and 1961 but shorter in 1971 The number f single intoxications was high but a small group of atients had ery high frequency of recurrences.

It is well known that the frequency of acute intoxication has substantially increased in recent years and that such cases constitute an increasing proportion of patients admitted to the emergency units of departments of internal medicine (1 5 6, 7 8 9 12) Swedish investigations have been reported either from university hospitals or from departments for intense therapy which may imply that the series are to a certain extent, selected We therefore thought it worthwhile to assess the frequency and tendency in an unselected material at the department of internal medicine of a large non-teaching city hospital

The investigation which compares the years 1951 1961 and 1971 regarding admissions because of acute intoxication to the Department of Internal Medicine of the Central Hospital of Helsingborg, was retrospective For technical reasons it did not include outpatients Acute intoxication is here to be understood as intoxication with any agent taken in an attempt to commit suicide or to get intoxicated, or as an act of impulsiveness. Cases of occupational intoxication and inad ertent intoxication with therapeutics are thus not included in the material

MATERIAL

The material which consisted of patients at least 15 years of age comprised altogether 366 cases. In 9 of these cases, 5 from 1961 and 4 from 1971 the records were incomplete and contained no information about loss of consciousness, earlier instances of intoxication or recurrences.

The number of cases of intoxication from other receiving areas were 3 (7 1%) in 1951 10 (10 6%) in 1961 and 14 (6.1%) in 1971 It is not known how many patients belonging to the area of Helsingborg got intoxicated outside the area and were admitted to other hospitals.

T get a more epresentative figure of the incidence in the receiving area, information about deaths outside hospital was also obtained from the police authorities concerning cases of acute intoxication not admitted t hospital.

Calculation of the incidence was somewhat complicated because the area catered for by Helsingborg Central Hospital was not the same in the three years studied. Thanks to cooperation with Helsingborg building authorities (Mr Sten Kristoffersson, Town Planning Centre of Helsingborg), however the total population and the age distribution of the population could be calculated with satisfactory degree of accuracy In 1951 and 1971 the receiving area, somewhat larger i 1951 consisted of the towns of Helsingborg and Höganäs with the surrounding areas. This indicates that about five-sixths of the population lived in medium-sized industrial town and port and the remaining sixth in a fairly densely populated rural district. I 1961 the hospital area consisted of only the town of Helsingborg (Table I).

The levels of significance were calculated by χ^2 analysis.

RESULTS

Frequency (Fig 1 Table I) In 1951 40 patients (19 women and 21 men) were admitted because of acute intoxication on altogether 42 a figure corresponding to 2.2% of

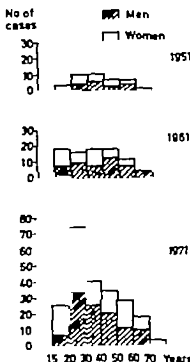


Fig 1 Number of cases of acute intoxication admitted to the Department of Internal Medicine 1951 1961 and 1971

of cases admitted to the Department of Internal Medicine In 1961 88 patients (39 women and 49 men) had been admitted on 94 occasions This represents 3 6% of the total number of cases is significantly higher than in 1951 ($p<$)The corresponding figures for 1971 were tients (113 women and 104 men) admitted 30 occasions or 7 6% of the total number of cases a highly significant increase from 1961 ($p<0$ 005)

Sex distribution (Fig 1) In 1961 intoxication tended to be more common among men than women. The difference was not significant, however and in 1951 and 1971 the figures were roughly equal for men and women

Age distribution (Fig. 1) Given in absolute numbers the age distribution in 1951 and 1961 showed a slight overrepresentation in the lower ages but in 1971 a marked preponderance of the 20–29 age group for both sexes Between 1961 and 1971 the age structure of the population in the receiving area had changed in that the per centage of individuals in the 20–29 age group had increased markedly

Incidence (Figs 2 3 Table I). The incidence in the receiving area was as mentioned above calculated on the basis of the combined material of inpatients and of patients who had died outside hospital. In 1951 5 persons (2 women and 3 men) with acute intoxication died outside hospital compared with 6 (2 women and 4 men) in 1961 and 37 (6 women and 31 men) in 1971 It was found that the incidence increased significantly both for men and for women between 1951 and 1961 ($p<0$ 001) as well as between 1961 and 1971 ($p<0$ 01 and $p<0$ 05 respectively)

The incidence in different age groups between 1951 and 1961 showed a significant increase for men in the 15–29 and the 40–49 age groups ($p<$ 0 05 and $p<0$ 01 respectively) and for women in the 15–19 and 30–39 age groups ($p<0.05$) From 1961 to 1971 the increase was significant for women in the 20–29 ($p<0$ 01) and for men in the 30–39 age group ($p<0$ 05)

Time of intoxication The distribution of the cases of acute intoxication among the months of the year showed no characteristic pattern though the frequency was higher in January in all 3 years. Neither did the frequency of intoxication vary notably with the day of the week

Intoxicants used (Figs. 4 5) In 1951 the most common intoxicants used were barbiturates (74% for women and 62% for men) In that year alcoho intoxication occurred in 21% of male and 4% o female cases In 1961 the use of barbituric acid preparations fell to 50% for women and 30% for men while the abuse of alcohol by men increased significantly from 1951 (from 21% to 36% $p<$ 0 05) In that year also other intoxicants began to appear though not very often However intoxica tion with meprobamate was noted in 19% of fe male and 6% of male cases In 1971 the picture had changed with a variety of intoxicants used al

Table I *Survey of results*

	1951	1961	1971
N of cases	42	94	230
% of all admissions	2.2	3 6	7 6
No. of fatal cases outside hospital	5	6	37
No of inhabitants ≥15 y in the receiving area	87 834	61 594	101 30
No of cases/100 000 inhabitants	54	162	264



Fig. 2. Incidence of acute intoxication in women (in-patients+patients who died outside hospital).



Fig. 3. Incidence of acute intoxication in men (in-patients+patients who died outside hospital).

most equally often. Alcoholic intoxication was, however noted in 34% of male and 18% of female cases. The latter figure indicates a significant increase from 1961 ($p<0.005$).

Fig. 5 gives the number of preparations per patient, with and without alcohol. It is seen that the number of cases with more than one intoxicant had increased substantially by 1971 and the total number of preparations per number of cases was significantly higher in 1971 (405/230) than in 1961 (131/94) and in 1951 (56/42) ($p<0.025$ and $p<0.005$ respectively).

Days in hospital (Fig. 6). Th number of days spent by the patients in hospital was roughly the same in 1961 as in 1951 but lower in 1971. Thus on day 4, i.e. 3 days after admission, 6.% of the patients wer still in hospital in 1951 compared with 67% in 1961 and 27% in 1971. The median values of the spell in hospital were 5, 4 and 2 days respectively.

Loss of onscio ne frequency of loss of consciousness tended to be lower in 1961 (41%) than in 1951 (55%) and 1971 (51%), but the differences were not significant. Loss of consciousness for more than 12 hours was noted in 29% of the unconscious cases in 1951, in 11% in 1961 and in 18% in 1971. The difference between 1951 and 1961 was significant ($p<0.05$).

Mortality. None of the cases of acute intoxication had been fatal i 1951 or 1961. It is, however possible that some patients who died shortly after admission had not been registered as in-patients. In 1971 there were 2 deaths (0.9%) and for that year there is no reason to suspect that any deaths shortly after admission had not been recorded as such. These figures may be compared with the number of deaths outside hospital: 5 in 1951, 6 in 1961 and 37 in 1971.

Previous intoxication or recurrence. For practical reasons the occurrence of previous intoxication or recurrence was assessed only for 1961 and 1971.



Fig 4 Frequency distribution of intoxicants used. Carbon monoxide intoxication was caused by coal gas.

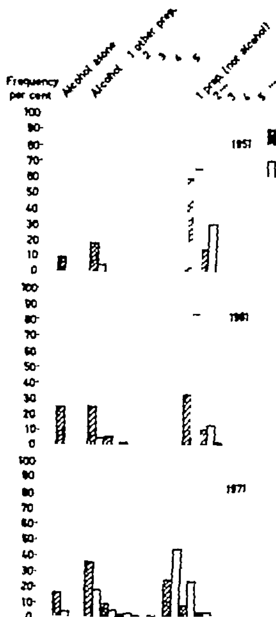


Fig 5 Frequency of intoxicants per patient with or without alcohol.

Sixty patients (72 %) of the 1961 material had neither previously nor during the subsequent observation period i.e. 11 years, had any further episode at our hospital. Of the remaining 23 patients 11 had only one more episode, while the other 12 (14 %), 4 women and 8 men had been admitted on altogether 98 occasions i.e. 8.2 times per patient.

Of the 1971 material 158 patients, 74 % had been intoxicated on only one occasion. Of the remaining 55 patients 34 had one further episode, while the remainder 21 patients (10 %) 7 wome and 14 men had been admitted on altogether 9 occasions i.e. 4.3 times per patient.

In the 1961 material recurrences were twice a common after 1962 as during 1961–62.

If only recurrences during the year of investiga tion and the following year are considered 10 c the 83 patients in the 1961 material had 18 re lapses while in the 1971 material 21 patients o 213 had 28 relapses The differences were not sig nificant.

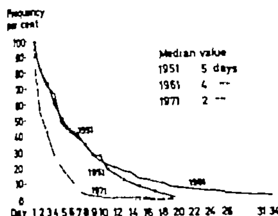


Fig 6 Spell in hospital given in cumulative curve.

DISCUSSION

The figures above for the frequency of admissions because of intoxication in 1951 1961 and 1971 can most probably be regarded as representati e of *such cases in the population in the receiving area* during the years in question. The number of pa tients from other regions was not ery large, and persons from the receiving area of Helsingborg Central Hospital are not admitted to other hospitals because of acute intoxication.

The tendency in the present investigation which spanned a period longer than that in earlier publications, confirmed the results in previous reports both from Sweden and other countries (1 5 6 7 9 11 12) This tendency i.e. a large increase in the number of cases of acute intoxication, especially in recent years was well illustrated by the fact that while every 50th admission in 1951 was because *of* acute intoxication, this was the case for every 30th in 1961 and for every 14th in 1971

The over-representation of individuals in the 20–29 age group in the 1971 hospital material was explained for men by a change in the age structure of the population in the receiving area, but for women there was also an increased incidence in this age group A tendency to an increase in the number of intoxication patients among younger people has also been reported by Smith and Davison (12) and Smith (11) and in an investigation of the mortality from acute intoxication at Sodersjukhuset, Stockholm, between 1956 and 1965 Thorstrand (13) found an increase in the mortality in the younger a after 1960

In most published investigations of intoxication women are predominant (1 5 9 10) though Fagher ( ) reported no significant sex difference in his material and Wilhelmsen et al (14) found men to be more common in most age groups In the present material there was no significant dif ference for the hospital patients but the increase in the number of fatal cases outside hospital was combined with an increased male dominance

As regards the intoxicants used the development was characterized not only by a change from a pronounced barbiturate dominance to a much wider variety of agents, *but also by an* increased occurrence of alcohol first for men but later also for women, and by an increasing frequency of intoxication *with more than one* agent. *The in*crease in frequency of intoxication with more than one poison, with or without alcohol has also been observed in other investigations (2, 5 6 7 9 10). Thorstrand (13) also found an increase in the frequency of intoxication with more than one *agent in fatal cases It is noteworthy that intoxica* tion with bensodiazepines was not more common despite the wider use of these drugs in recent years (3 4)

The recent development with an increasing number of potential poisonous substances and an increased occurrence of multi-intoxication with a risk of interaction, has made the management of acute intoxication more difficult, both from diagnostic and therapeutic points of view

In their Uppsala investigation Hedstrand and Ljungström (5) found a certain seasonal variation with the highest frequency in May while in the present investigation as in a Gothenburg material (14) *only small but not significant* monthly variations could be demonstrated In the Uppsala investigation the frequency was also somewhat higher at week-ends. In our investigation the frequency was not higher during week ends, which was a surprise to the investigators for one of the reasons why the present investigation was undertaken was the strong impression that cases of acute intoxication seen in our emer gency unit were more common at week-ends The reason why this impression could not be confirmed by the investigation was probably that the latter included only inpatients.

The frequency of deaths in our 1971 hospital material was roughly the same as in vestigations (2, 7 13) It is remar

total number of deaths outside hospital did not increase between the years 1951 and 1961 while from 1961 to 1971 it increased by 500%

The shorter duration of hospital care in 1971 than in 1961 is striking. It cannot be explained by an increase in the number of milder cases, since the severity of intoxication had if anything tended to increase A possible explanation might however be that intoxicants with a faster elimination are now more common. A contributory factor might also be that for psychological reasons psychiatric after-care was sometimes given formerly at the Department of Internal Medicine

The prognosis was good for most of the patients as about three-fourths of them had only one episode of acute intoxication while it was bad for a small group somewhat more than 10% who had a high frequency of episodes with recurrences even after several years. One may wonder whether it would not be possible to prevent intoxication in the first group by enabling the patients to contact some suitable social or medical organization which might help them to solve their problems But probably such a procedure is often not possible, because in most of the cases the intoxication was apparently an act of impulsiveness. Therefore most often the only possible prophylaxis for intoxication seems to be to limit the availability of potential poisons

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ATTEMPTED DRUG SUICIDE IN A SWEDISH CITY

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Abstract A study has been made of the number of attempted suicides with drugs and toxic gases seen in a medical department of general hospital during 1968–70 The material consisted of 2002 patients over 15 years of age who made 2663 suicidal attempts. The greatest numbers occurred in younger age groups and there was slight predominance of women About 20% of the men and 17% of the women made more than one attempt during the period. Barbiturates and benzodiazepines were the most frequent drugs used During 1969–70 there was sharp increase of drugs such as methaqualone and dextropropo yphene The mortality rate for patient alive at admission was low 0.4% The investigation lso included maximum 5-year follow-p which showed that 4% of the patients who after attempting suicide by intake of drugs in 1968–70, later made another and successful suicidal attempt. There was often no correlation between the seriousness of the latest attempt from somatic point of iew compared with the risk of later death through drug intake.

In recent years there ha e been many reports on the increase of patients admitted to hospitals because of attempted suicide with drugs (7 8, 11 14). As will appear from the present study these patients are a great problem for the Medical Department of a hospital The crude rate was three times greater in 1971 than it was ten years ago and the cases constitute 7–8% of all patients annually admitted to the Emergency Ward

MATERIAL

Malmo is an industrial town of about 250000 inhabitants. There is only one hospital. The Medical Emergency Ward provides treatment for patients who have taken drugs or inhaled toxic gases This investigation concerns all patient admitted during 1968–70 It has not been possible to include suicidal attempts by active methods Mere accidents and ingestion of alcohol alone are not counted. The age limit is 15 years The material is completely unselected Even patients with only slight symptoms are included, but all were hospital because of deliberate intake of drugs. The study is retrospective all data being derived from records vailable at the Medical Department and in many cases also from the Department of Psychiatry The patient remain in the Emergency Ward until awak and considered to be out of danger Thereafter as matter of routine all are remitted to psychiatrist in the hospital

One aim of this examination was to find out whether there has been an increase in the course of three years For this purpose part of the study covers each year another part all three years together Through autopsy records at the Institute of Forensic Medicine all completed suicides in the city of Malmo during the same time have been recorded. It has also been possible to trace such patients in the material as have later died, either by suicide or disease The maximal follow up time is five years. The autopsy frequency in the region is very high about 90% (13) which applies equally to people dying in and out of hospital.

RESULTS

Table I shows the number of patients and the number of attempts for the years 1968–70 and totals for the three years together During 1969 there was an increase by more than 200 attempts but this increase did not continue during 1970

Fig 1 shows the age and sex distribution of the patients compared with that of the inhabitants of Malmö On the whole attempts were more frequent in the younger age groups and n the 15–19 age group there were twice as many girl as boys.

Many of the patients made several attempts in the course of the investigation. Table II shows the number of attempts per perso There was no significant difference between the sexes in the percentage of repetition Of the total number of patients 21% were known to ha e made attempts before 1968

Toxicological serum analysis was not out regularly In many cases the ty

Table I *Number of patients and suicidal attempts during 1968–70 in Malmö*

	No of pats.			No of attempts			
	Men	Women	Total	Men	Women	Total	No. of deaths in hospital
1968	793	330	623	363	393	756	
1969	419	426	845	508	486	994	5
1970	390	395	785	467	446	913	2
Total	968*	1 034*	00**	1 338	1 325	663	9

These figures are les than the sum of patients in the column because the *same patient* sometimes made several attempts.

Table II *Distribution of attempts during 1968–70*

	No of attempts										
	1	2	3	4	5	6	7	8	9	>10	No of repeaters
Men	770	126	35	1	5		3	0	4	2	198 (20%)
Women	857	110	43	13	4	4	2	0	1	0	177 (17%)

taken therefore remained unknown Very frequently more than one drug was taken at the same attempt Relatives or friends could often give information sometimes bringing empty bottles of pills A rough estimate of the drugs taken shows that barbiturates are still very common though there was a slight decrease in 1970 Instead there was a sharp increase during 1969–70 of such drugs as methaqualone benzodiazepines and dextropropoxyphene Intoxications with tri- antidepressants and phenothiazines did not in number Inhalation of carbon monoxide frequent in 1970 with only 11 cases com- 1 to 20 in 1968 Still there are about 50000 omes in Malmö with gas instead of electricity for cooking Ingestion of alcohol together with drugs was recorded in 47% of the male patients and in 18% of the female

Therapy mainly followed the lines described by Clemmesen (2) Thus it was essential to observe the patients for signs of circulatory or respiratory failure Gastric lavage was used if the patient was awake and admitted within the first two hours after drug intake Such patients comprised 12% of the total Forced diuresis was often practised but not haemodialysis or peritoneal dialysis No central stimulants were used During the period of investigation 114 patients (4%) needed respirator treatment. There was no significant difference between the years as regards percentage of respirator treatment. The drugs used by the patients with respiratory failure followed the same pattern as described earlier for drugs in general There was a decrease of barbiturates but no change in tricyclic antidepressants It was more common in 1969–70 to mix various drugs at the same attempt, and new drugs such as methaqualone and dextropropoxyphene became more frequent

The frequency of somatic complications was very low 38 patients had infections, mostly pneumonia 6 peripheral paralysis and 33 other complications Only 9 patients died in hospital in connection with self-poisoning the mortality rate being 0.4% Two of these patients died from inhaling carbon monoxide both being in a very bad condition when admitted with respiratory failure. Three patients died very soon after admission from intake of a tricyclic antidepressant dibenzepine (Neodalit®) This drug was introduced in Sweden

Table III *Suicidal deaths outside hospital (max. 5-year follow-up)*

Methods	Men	Women	Total
Drugs	44	22	66
Carbon monoxide	4	5	9
Hanging	3	1	4
Drowning	3	1	4
Jumps		2	4
Firearms	2	0	2
Total	58	31	89

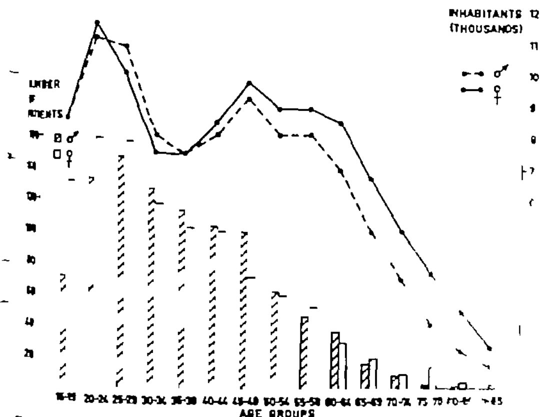


Fig. 1. Age and sex distribution of suicidal attempts in Malmö in 1968–70

in 1968 and all three deaths occurred within six months. The other four patients died from combinations of barbiturates, meprobamate and alcohol.

Of the total number of patients 56% of the men and 52% of the women had been treated at the Department of Psychiatry before the episode under study. In connection with the actual attempt 16% of the men and 19% of the women were admitted to the Department of Psychiatry as in-patients.

Table III shows the result of the 5-year follow-up. There were 89 patients (4%) who, after attempting suicide by intake of drugs i 1968–70 made another and successful suicidal attempt. Active methods were seldom used. The average age at death was 45 years. All of the patients were in contact with a psychiatrist, though some of them irregularly. 11 had committed suicide during their stay in the Department of Psychiatry. An analysis of the psychiatric diagnoses is beyond the scope of this study, but as many as 56% of the men in this group were known to be chronic abusers of alcohol and drugs. They were also among those who made the most attempt.

During the same period of follow-up 40 of the patients (%) were found dead from disease or accident, of whom 21 from atherosclerosis, 11 from malignancy and 8 from accidents. The average age at death was 58 years. Among those who died from accidents there might be cases of suicide, though naturally impossible to confirm.

The total number of suicides in Malmö during 1968–70 is shown in Table IV. As noted, the numbers for each year were fairly constant. Half of the cases committed suicide with drugs, men more commonly than women, the ratio being 1. Fig. show the age and sex distribution of suicides. The peak is between 40–54 years of age whereas for suicidal attempts it i between 20–34 years (Fig. 1).

DISCUSSION

As shown in thi study, the number of patient admitted to hospital after intake of

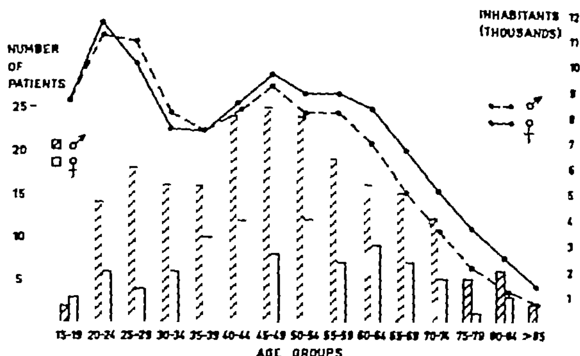


Fig 2. Age and sex distribution of suicides in Malmö in 1968–70.

great problem to a Medical Emergency Ward In Malmö there are about ~3 patients of this type a day per year Although many of them have only taken a small amount of tablets it is often difficult, on admission, to assess the gravity of self poisoning. All cases require careful observation sometimes the facilities of an intensive care The mortality rate for patients admitted is (0 4%) but, as the follow-up shows there considerable risk of another suicidal attempt fatal outcome (5) To judge the intention underlying every suicidal attempt with drugs is very difficult and is the concern of the psychiatrists It has often been discussed whether "suicidal attempt" is the right term for poisoning (4) The present investigation shows that, even if the patients with a small intake of drugs are included, there is a 5-year mortality rate of 4% To compare the results of follow-up studies is difficult since the selection of materials is so different, but the above mentioned number must be considered high (1 3 6 9 10, 12). There is often no correlation between the seriousness of the latest suicidal attempt from a somatic point of view as compared with the risk of later death through drug intake. In the present study this is true of many patients

Table IV *Suicides in Malmö during 1968–70*

	1968		1969		1970	
Methods	Men	Women	Men	Women	Men	Women
Drugs	36	19	38	17	35	19
Carbon monoxide	11	6	13		10	1
Hanging	13	4	9	1	18	4
Drowning		4	4	1	3	6
Jumps	3	0		0		0
Cuts	1	0		1	4	2
Firearms	3	0	3	3	4	1
Total	69	33	71	25	76	33
	102		96		109	

classified as chronic alcoholics who had been treated on many occasions for self-poisoning with relatively mild symptoms but who were found dead from drugs outside the hospital at follow-up. The same could also be said about some young patients whose self-poisoning was considered an act of impulse or demonstration with no real suicidal intention. They died later because they managed to get hold of more dangerous drugs for instance methaqualone or tricyclic antidepressants.

It is therefore essential that doctors should be more careful when prescribing hypnotic and sedative drugs. Between two drugs with similar therapeutic effect the less toxic one should be chosen, and always in small amounts. Also the drug companies should be asked to make smaller packages and perhaps more so-called "strips" making it more difficult to take a handful of tablets at once. It is also very important to make the public aware through the press, TV and radio of the great dangers involved in the use and abuse of drugs. People should be taught not to ask for tablets every time they are faced with problems in life.

Such measures of course would not put an end to suicidal attempts, but they might stop the increase particularly if more psychiatric help was offered to chronic abusers and repeaters.

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BOOK REVIEW

Radioimmunoassay and saturation analysis Brit med Bull vol. 30 o 1 Jan 1974 Published by the British Council London, 102 pages. £2.50

This last volume of the excellent Medical Bulletin treats a subject that is of very great interest both to the clinician and to the biochemist. Exactly one-half of this number is devoted to purely technical questions concerning the methods It contains a wealth of information for the workers in isotope laboratories as all the authors are specialists in this field Many ha e contributed actively to the development of the new science that has developed explosively since the first publication by Yalow and Berson less than 15 years ago The reader will find that the applicability of the new technique has become very wide in recent years. This has allowed the determination of almost homeopathic concentrations of many important substances, and the scale usually moves in the picogram range.

I shall not discuss the technical part, as this is rather far from clinical medicine I the latter part, however the clinician receives concentrated and up-to-date information not only about the technical potentialities but also a review of clinical facts in the rapidly xpanding province of internal medicine that has to do with protein and polypeptide molecules active at low concentrations.

The oncologist will find an excellent presentation of tumour-associated antigens by on of the great authorities in this field K. D Bagshawe from London There is much controversy regarding the diagnostic importance the antigens, that were first prepared from fetal ,, for the diagnosis of different carcinomas. This field is in rapid flux and it is necessary for every thing doctor to follow the developments. The re iewer notes with pleasure that the pioneering ork of K. O Pedersen in Uppsala, when he first described fetuin as fetal protein, is recognized Most modern presentation of this subject seem to have forgotten this discovery that was made already in 1944 The biological problem of fetuin function is still obscure but its practical importance for the diagnosis of malignant hepatoma has become very great It seems certain that a large number of other proteins, some of them of fetal type will be connected with malignancy and the presentation of placental phosphatase and lactogen is one example. W have had xperience with another type of liver phosphatase connected with malignant tumours that may perhaps become diagnostically important A paper on this subject has appeared in this journal (Axelsson et al. March 1974).

One of the most exciting stories in modern medicine is the intimate collaboration between clinical observers and biochemical analysers in the field of gastrointestinal hormones. This chapter alone makes the book w ll worth its price

The reader will find an excellent review by S. R. Bloom who has himself contributed so much to this development. The history of the first substance that was given the designation hormone by Bayliss and Starling already in 1902 is rather paradoxical The work on secretin has lasted for three quarters of the century and an enormous number of different hormones were isolated and synthetized before this first member of the family became definitely purified and analysed. At the same time large number of smaller polypeptides from the GI tract hav also been studied and the recent excellent methods for sequence studies of amino acids have given s an insight into the similarities and differences between the members of a whole family of molecules. It has become clear that secretin and glucagon from the pancreas and from the gut, on the one hand, as well as the gastric inhibitory peptide (GIP) and the vasoactive intestinal peptide (VIP), on the other have partial structures in common. The two last named substances are still not well investigated from the linical point of view but it seems clear that the VIP is identical with the diarrhea substance that causes so-called pancreatic cholera. It is probable that this substance as well as the GIP a factor counteracting gastric hypersecretion may have clinical importance.

Two substances seem to have completely different activity but still part of their molecule is the same This applies to cholecystokinin that has been studied extensively by Jorpes and Mutt at the Karolinska Institute in Stockholm. It is probable that this hormone will become of great importance also to the clinician. This is true of the other hormone gastrin as has already been amply proved by clinical gastroenterology

The new RIA techniques (radioimmunoassay) will have an immense importance for the clarification of problems in this field It is important to remember that many of these substances were first obtained from pancreatic tumours and that the clinicians Zollinger and Ellison were pioneers in the field The new methods for determining low concentrations of these substances rest on the fact that they have been prepared in a completely pure form. One of the leading schools has been headed by Jorpes and Mutt who prepared highly purified secretin for clinical use already 30 years ago. In spite of this absolutely pure secretin is comparati ely recent will be available also for the determination of th hormone

I have quoted all these facts in order to illustrate the intimate relation between clinical observation on patients biochemical analysis of active substances and application of the latest supersensitive methods for quantitative determination.

The book i strongly recommended to all students and doctors who are interested in the fundamental biological problem at the basis of disease

Jan G Waldenström

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INTRABRONCHIAL ASPIRATION OF METALLIC MERCURY

Kurt Einarsson Kjell Hellström Andrejs Schütz and Staffan Skerfving

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Abstract By accident 60-year-old man aspirated about 20 g metallic mercury into the bronchial tract. Most of the metal disappeared within one year and there was no evidence of acute or chronic mercurialism. Repeated analyses demonstrated elevated levels of mercury in blood and urine However the combined results from these studies indicate that most of the mercury was eliminated with the sputum or possibly by the breath

Long-term inhalation of elemental mercury vapour is the most common route of chronic mercury poisoning, characterized by symptoms from the central nervous system and occasionally by tubular kidney damage (9). Massive exposure to such vapour may cause acute symptoms of intoxication affecting the nervous system the lungs (bronchitis bronchiol'tis and pneumonitis) and the gastroin testinal tract.

Metallic mercury has been found to accumulate intrapulmonarily after accidental intravascular injections of the metal A minor degree of embolization does ot appear to be associated with general mercurialism or local effects on the lungs and the emboli disappear gradually (1 7) Extensive pulmonary embolization of mercury globules has been accompanied by chest pain dyspnoea, fever and as late sequelae bronchiectasis and lung abscess (1 2, 6).

Metallic mercury has also been aspirated into the lungs in a few patients during the insertion or removal of intestinal tubes (7 1 13 14) Such an accident occurred recently at Serafimerlasarettet The present paper reports on the follow-up of the patient a study which included repeated measurements of mercury n blood and urine

CASE REPORT

A 60-year-old lectrician was admitted because of steatorrhoea. He had been addicted to alcohol for many years. Diabetes mellitus and steatorrhoea secondary to hronic pancreatitis developed when he was 50 years old and required treatment with insulin and pancreatic enzymes (Pancreozym). In addition he was prescribed Diphydan because of epilepsy

On admi sion his condition was fairly good. There was no evidence of heart or respiratory insufficiency anaemia or malnutrition (Table I). Approximately 20 g glucose/24 h was excreted in the rine and the excretion of fat in faeces was elevated.

Duodenal intubation with single lumen tube was instituted as part of the investigation of the steatorrhoea at the tip of the tube there was rubber bag containing about 20 g mercury to facilitate the passage into the intestine Wh le the bag was passing the pharynx the patient experienced pain and started to cough. Upon withdrawal of the tube it was found that the bag had ruptured and that most of the mercury had disappeared After some minutes the patient stopped coughing and felt no discomfort. X-ray showed mercury throughout both lung fields, especially on the left side (Fig. 1). During the next few days he was treated with intermittent postural drainage forced coughing and suction of the tracheobronchial tree Minor amounts of mercury-like material was recovered the sputum. However the effect of this therapy was too slight to be detected from repeated X-ray examinations of the chest.

The patient was perfectly w ll in the weeks following the aspiration and there were no symptoms of damage to the central nervous system, lungs, kidneys or gastrointestinal tract.

The patient wa readmitted on several occasions du ing the subsequent year Repeated chest X-ray examinations showed that about half of the mercury had disppeared from the lungs after 4–5 months, with further improvements 6 month later (F g ?) On comparing the X-rays obtained on different occasion it seemed that some of the mercury particles had disappeared completely whereas others were unchanged.



Fig 1 X-ray of the k immediately after the aspiration.

No signs of mercury intoxication were evidenced by the clinical picture and con entional laboratory tests (Table I). An EEG showed no abnormalities.

METHOD

minations of m rcury in blood and urine

s blood was collected in acid-washed tubes con ing heparin Blood cells and plasma were separated by centrifugation at 200×g for 10 min. Urine was collected in acid-washed bottles for 24-hour periods.

Total mercury in blood cells plasma and urine was analysed by the method of Schütz (8) The samples were combusted the mercury wa absorbed in potassium permanganat solution which was reduced by hydroxylamine whereafter elemental mercury vapour was liberated with stannous chloride and determined by atomic bsorption spectrophotometry The limit of detection was 1 ng/g. The coefficients of correlation of repeated analyses of blood cell samples containing 5 and 100 ng/g were 5 and 1% respectively

RESULTS

Samples of plasma, blood cells and urine were repeatedly analysed for mercury (Fig. 3) The plasma level reached 130 ng/g during the first 7 days and was about 50 ng/g a couple of weeks later It then decreased only slowly the values recorded after one year being about 30 ng/g. Although it varied greatly the concentration of mer cury in the blood cells showed the same general trend as in the plasma. The level of mercury in the blood cells was always about equal to or

Table 1 *Laboratory findings before and one year after the intrabronchial aspiration of metallic mer cury*

	Before	After
Blood		
Hb (g/100 ml)	14 5	14.9
WBC/mm³	4 200	3 000
ESR (mm/h)	38	45
SGOT (U/ml)	4_	42
SGPT (U/ml)	34	45
Alkaline phosphatase (U/ml)	71	58
Creatinine (mg/100 ml)	0 7	0 7
Urine		
pH	5	6
Protein	Neg.	Neg.
Sediment	Normal	Normal



Fig 2 X-ray of the lungs one year after the aspiration.

lower than that simultaneously recorded in the plasma. There were large day-to-day variations in the urinary excretion of mercury which averaged 50 μg/day during the study There was no obvious relation between the concentration of mercury in blood and the amount of the metal excreted in the urine

DISCUSSION

The elevated concentrations of mercury in plasma blood cells and urine of our patient indicated a slow but sustained absorption of the metal Although the uptake of metallic mercury from the gastrointestinal tract is low (10) it is possible that part of the mercury absorbed during the first days may represent material swallowed by the patient. The mercury recovered later on in blood and urine most probably originated from the lungs.

The levels of mercury recorded for plasma and blood cells during the first week after the aspiration were similar to those found in workers exposed to elemental mercury vapour (3). After one year the level in blood cells was slightly above the range of an ordinary Swedish population (11) but the plasma concentration was elevated about 10 times.

The amount of mercury excreted in the patient s urine was low compared with the findings in occupationally exposed workers (3) and only slightly higher than that encountered "normally" (4)

Mercury is eliminated from the body mainly with the urine and faeces Upon exposure to inorganic mercury the urinary excretion usually exceeds the faecal (5). The patient s urinary excretion of mercury averaged 50 μg/day indicating that the total amount eliminated by this pathway during one year amounted to about 20 mg Since the faecal excretion may have been even less, it appears that the combined urinary and faecal excretion of mercury amounted to only a few % of the total amount eliminated according to the evidence from changes in the lung X-rays.

Some mercury may have been retained in extrapulmonary tissues particularly in the kidneys and the central nervous system Judging from pr

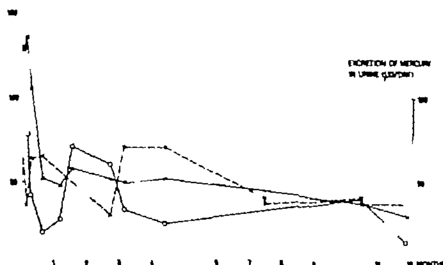


Fig. 3. Recovery of mercury in plasma (x—x), RBC (O—O) and urine (x x) at various times after intrabronchial aspiration of mercury

observations of subjects exposed to mercury the amount accumulated in this way should hardly exceed a few hundred mg (5)

The indirect evidence referred to above thus indicates that the patient eliminated most of the mercury directly from the lungs Metallic mercury is highly volatile at body temperature and it is quite conceivable that part of the metal disappeared with the breath Analyses of a few specimens of sputum several months after the aspiration showed that substantial amounts of mercury were eliminated by coughing. The observation by X-ray ... some of the intrapulmonary mercury particles ... to be unchanged whereas others had dis-...ared completely between two examinations may be taken as evidence that elimination with sputum was a more important route than the breath

To our knowledge only four cases of intrabronchial aspiration of metallic mercury have been published. A 48 year-old man with Hodgkin s disease aspirated about 6 ml (80 g) mercury (14) Some days later he showed weakness fever abdominal pain bloody diarrhoea leucocytosis albuminuria and circulatory collapse Therapy with dimercaprol (BAL) was started but the patient died mainly because of massive endobronchial haemorrhage

Another patient (age 34 years) described by Schulze (7) ingested metallic mercury in a suicidal attempt and aspirated unknown amounts of mercury while vomiting. Necrotizing gingivitis alveolar pyorrhoea and X-ray signs of pneumonitis developed within a few days The patient recovered and during the next five years there was no evidence of chronic mercurialism even though large amounts of mercury remained in the lungs Two other patients a 79-year-old man (12) and an 11 year-old boy (13) aspirated about 5 ml (70 g) mercury into the bronchial tree The follow-up studies revealed no signs of acute or chronic mercurialism In the old man the mercury disappeared within two years as evidenced by the X-rays.

The present patient is thus the third in whom intrabronchial aspiration of substantial amounts of mercury led to no detectable complications of acute and chronic mercurialism. This absence of toxic effect in our patient is compatible with the rather small amounts of mercury recovered in blood and urine However the levels of mercury in blood and urine are considered for several reasons (3 5) to be of limited value as an index of the risk of poisoning by inorganic mercury especially when exposure is brief

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Table I *Methionine and homocystine in plasma (μmol/ml) and urine (μmol/24 h) before and after treatment*

	Before treatment	After treatment	Normal range
Plasma			
Methionine	0 089	0 097	0 011–0.030
Homocystine	0 042	0 0080	0
Urine			
M thionine	75 4	41.8	70–81
Homocystine	783	71 7	0

Cyanide-nitroprusside reaction and thin-layer paper and column chromatographies were also performed as previously described (11)

CASE HISTORY

Our patient was a 22-year-old married woman with an earlier abortus provocatus in the 3rd month and ex perience of four pregnancies, all ending in partus provocatus after about 7 months due to death of the foetus. Autopsy of two of the foetuses revealed nothing abnormal.

The patient was referred to hospital with moderate hypertension Routine ophthalmoscopy showed luxation of the eye lenses and this caused an xamination for homocystine in urine A pronounced positive reaction of cyanide-nitroprusside was revealed, which by means of thin-layer and paper chromatographies was shown to be caused by great amounts of homocystine in the rine.

/ means of column chromatography we found 86 l homocystine in the 24-hour rine and by the ie technique a methionine concentration of 0,089 mol/ml plasma (i. about 3 times the normal amount) Methionine load test with 100 mg methionine/kg b wt. was exceedingly abnormal Liver biopsy showed steatosis of the liver in a moderate degree EEG showed dysrhythmia as seen in epileptics.

All the following examinations proved to be normal. Hb leucocyte and differential count serum electrolytes, total cholesterol non-esterified cholesterol, phosphoru lipid, fatty acids, triglycerides, serum aspartase transaminase serum LDH prothrombin basic phosphatases, serum bilirubin glucosuria, ECG chromosome analyses (Philip) X-ray examinations of heart and lungs, of kidneys by i.v. pyelography and aortography and of the bone structures, examination of the eye grounds and of the liver biopsy concerning the content of copper in the liver tissue

Tentative treatment was initiated partly with folic acid pyridoxine and vitamin B_{12}, one by one or in combinations and partly with a diet poor in methionine combined with the above mentioned vitamins. During this treatment we succeeded in reducing the precipitation of methionine in urine, while the concentration of methionine in plasma remained almost unchanged. The concentration of homocystine in urine was partly reduced. Furthermore the patient was given an extra supply of cystine and homoserine. The most effective treatment seemed to be a diet poor in methionine combined with pyridoxine and folic acid.

RESULTS

Table I shows the concentrations of methionine and homocystine in plasma and urine before and after treatment. It appears that during the period of treatment with pyridoxine 250 mg×4 folic acid 20 mg×4 cystine 250 mg×2, and a diet poor in methionine a solid reduction of the precipitation of homocystine in urine was observed together with a significant decrease in plasma homocystine while the precipitation of methionine urine was nearly halved, and the plasma methi nine concentration remained unchanged

The results of the coagulation analyses a shown in Table II Without treatment the patie had a decreased factor V content and the fibrin

Table II *Coagulation and fibrinolysis*

	Before treatment	During treatment
Clotting time (min)		
Glass	12	12
Plastic	24	26
Recalcified citrated plasma (sec)	155	88
Bleeding time (min)		
Duke	2	3
Ivy	10	10
Platelet concentration ×10³/μl	247 000	229 000
Platelet adhesiveness (Hellem) (%)	23	19
Prothrombin consumption (%)	0	0
Factor VIII (%)	109	118
Factor XI (%)	69	64
Factor XII (%)	104	80
One-stage prothrombin (sec)	16.0	15.8
Factors II+VII (%)	64	77
Factor V (%)	43	87
Fibrinogen – EACA (g/100 ml)	0 23	0.74
Fibrinolytic activity on un heated plates (mm²)		
Citrated plasma	0	0
Euglobulin	32	46
Euglobulin clot lysis (min)	200	250
FDP (μg/ml)	0	0
Platelet aggregation (Born)		
ADP	Normal	Normal
Adrenalin	Normal	Normal
Collagen	Normal	Normal

gen content was 0.3 g/100 ml During treatment she had a normal factor V content and a high fibrinogen value. Moreover the values as to platelet adhesiveness platelet aggregation and the other components of the coagulation and fibrinolytic system both with and without treatment were normal

DISCUSSION

The previous literature postulates changes in the platelet adhesiveness platelet aggregation and the concentration of the Hageman factor in patients with homocystinuria facts that have not been confirmed by the methods used in our investigation. No other sign of hypercoagulability was found

Thromboembolic episodes are frequent in patients with homocystinuria but the basic mechanism of this tendency has yet to be disclosed It might be that the abnormal metabolism in these patients affects other mechanisms of importance for the development of thrombosis, such as the structure of the collagen or the subendothelial substances The clinical manifestations of the disease vary so much that we may venture the hypothesis that other factors than the primary biochemical defect (viz. the absence of cystathionine synthetase) may contribute to the final phenotype. These factors could then be either genetic and/or exogenous

Whether treatment would improve the prognosis of patients in whom cystinuria is complicated by thromboembotic episodes still remains to be proved.

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Congress Announcements

The First World Congress of Nuclear Medicine will be held in Tokyo Japan Sept. 30 – Oct. 5 1974 under the precidency of H Ueda, MD

The George von Hevesy Prize for Nuclear Medicine amounting to Swiss francs 10000 will be awarded again Unpublished scientific papers preferably in English accepted also in German and French on nuclear medicine can be submitted by authors up to the age of 40 Manuscripts not exceeding 8 type-written pages should be sent before Aug 10 1974 to Prof Dr W Horst Clinic of Nuclear Medicine University of Zürich Raemistrasse 100 Ch-8006 Zürich Switzerland

The II Congress of the International Rehabilitation Medicine Association will be held in Mexico City Mexico Oct. 27 – Nov 1 1974

Further Information. General Secretary of the Congress Dr A Tohen Apartado Postal 714-7 Mexico D F